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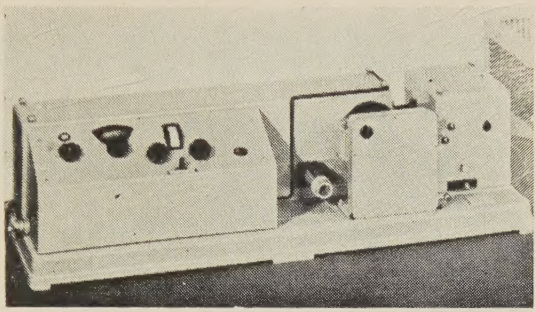

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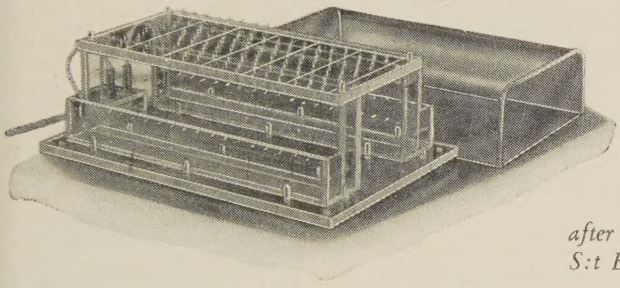

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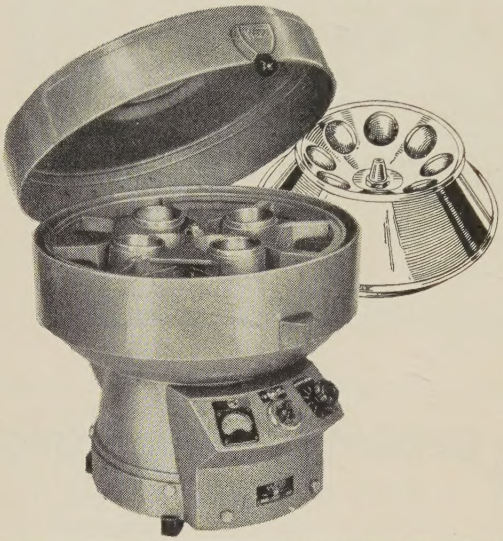

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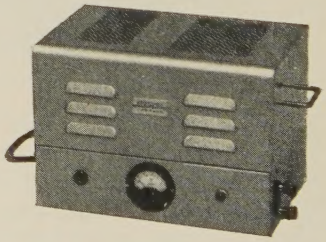

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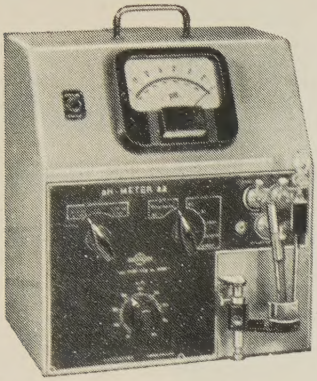

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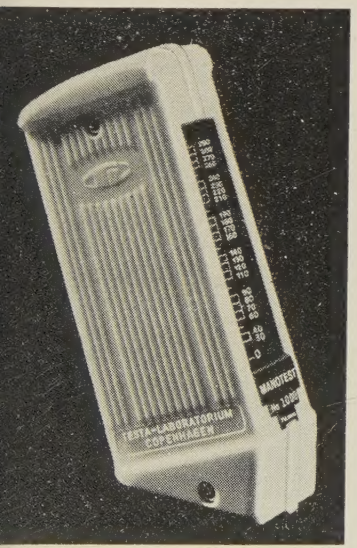

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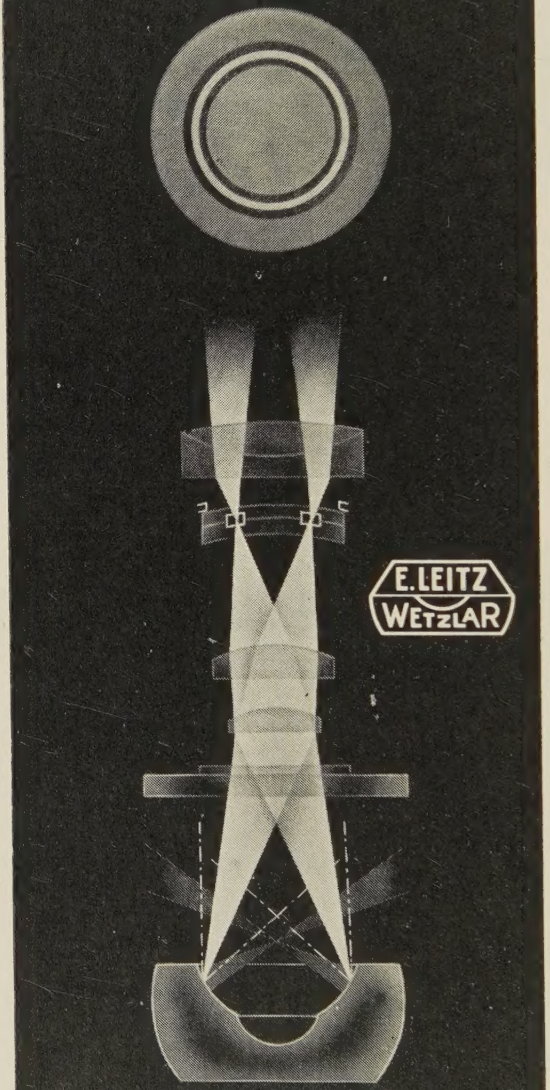

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ON THE VITAMIN B_{12} CONTENT IN FAECES

By W. J. KAIPAINEN, S. WALLÉN and G. TØTTERMAN[1]

From the Medical Department of Maria Hospital, Helsinki, and from the Second Medical Clinic and Department of Serology and Bacteriology, University of Helsinki, Finland

(Received for publication Nov. 2, 1953)

It has earlier been shown (Berk, Castle, Welch, Heinle, Anker & Epstein 1948, Lethell, Meyers & Neligh, 1948) that the faeces of patients with pernicious anemia contain abundant vitamin B_{12}. Similarly, it has been shown (Kaipainen & Tötterman, 1954) that the faeces of fish tapeworm anemia patients contain a considerable amount of vitamin B_{12}, varying within the limits of healthy subjects. The vitamin B_{12} in the faeces derives partly from food, in part is synthetisized by the intestinal bacteria, and can also be secreted by human glands.

It has been found that gastric juice (Ternberg & Eakin, 1949) is capable of binding vitamin B_{12} so as to make it inutilizable by bacteria. It has been assumed (Ternberg & Eakin, 1949) that the factor to which vitamin B_{12} is bound is the very intrinsic factor necessary for the resorption of vitamin B_{12} from the intestines. This factor, which is thermolabile, forms a thermolabile complex with vitamin B_{12}. Saliva is also capable of binding vitamin B_{12}. The saliva, however, has not been found, in clinical tests, to contain the intrinsic factor (Castle, Townsend & Heath, 1930). Several substances bind vitamin B_{12} (Bacon, Chow & Yamamoto, 1953). The binding power of gastric juice has been found to be absent in pernicious anemia patients and in the aged (Bacon et al. 1953). Its absence in the aged in general suggests that it is not an intrinsic factor. It has been remarked (Welch & Heinle, 1951) that it would appear unsafe, without good clinical evidence, to conclude that any binding phenomenon is to be attributed to the intrinsic factor.

The purpose of the present investigation is to study whether faeces contain vitamin B_{12} bound to some thermolabile factor from which it can be liberated by boiling. The faeces were taken from the following cases: a healthy subject, a worm carrier (Taenia saginata), a patient with pernicious anemia, three patients with pernicious fish tapeworm anemia and a patient with intestinal megaloblastic anemia (Siurala & Kaipainen, to be published).

[1] Lady Tata Memorial scholar.

Relation between free and bound (to some thermolabile factor) vitamin B_{12} in faeces, microbiological assay using E. coli strain 113—3.

Case	Diagnosis	Vitamin B_{12} content of 1 g dried and pulverized faeces	
		free	bound
1.	Healthy subject	0.25	0.5
2.	Carrier of Taenia saginata	0.12	0.25
3.	Pernicious anemia:		
	before treatment	0.06	0.5
	after treatment with B_{12}	0.5	0.25
4.	Fish tapeworm anemia:		
	before worm cure	0.25	0.5
	after worm cure	1.0	0.5
5.	Fish tapeworm anemia:		
	before worm cure	1.0	0.5
	after worm cure	1.0	0.5
6.	Fish tapeworm anemia		
	after worm cure	0.5	0.125
7.	Intestinal megaloblastic anemia	0.5	0.035

TECHNIQUE

The amount of free vitamin B_{12} in dried pulverized faeces was determined by the method reported in detail previously (Kaipainen & Tötterman, to be published).

To determine the so-called bound vitamin B_{12}, the free vitamin B_{12} had to be removed first. Mutant E. coli strain 113—3 (Diding, 1951) was employed for this purpose. The bacteria were cultivated in broth and washed with saline solution. The faeces solutions to be tested, sterilized by filtration, were poured in to the washed bacteria, the solution shaken vigorously, left to stand in an incubator for 2 hours, centrifuged, and the supernatant treated with bacteria washed in the same way. Finally B_{12}-free culture medium was added to the solution (Diding, 1951) of the following composition: 7.0 g of K_2HPO_4, 3.0 g of KH_2PO_4, 0.5 g of Na_3-citrate · $3H_2O$, 0.1 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of $(NH_4)_2SO_4$, 10.0 g of glucose (autoclaved separately), and 1000 g of water. The same E. coli strain was grown on this solution, centrifuged and culture medium added until the growth of E. coli strain 113—3 in the solution was slight or completely inhibited. The faeces solution obtained was passed through a Chamberlain filter. The sterile filtrate was boiled for an hour after which the amount of vitamin B_{12} liberated on boiling was determined by the 2-fold serial dilution method using E. coli strain 113—3 and the above culture medium.

RESULTS

The results of the investigation are given in the table. It shows that the amount of so-called bound vitamin B_{12} in the faeces varies to some extent but not as much as the amount of free vitamin B_{12}. The amount of bound vitamin B_{12} seems to be independent of the amount of free vitamin B_{12} present in the faeces samples. A remarkably small

mount of bound vitamin B_{12} was found only
the case of intestinal megaloblastic anemia.
binding power and intrinsic factor are iden-
cal the faeces of a pernicious anemia patient
ight be expected to contain less bound vita-
in B_{12} than the faeces of a fish tapeworm
emia patient as the latter have the intrinsic
ctor, as has been clinically shown earlier
Hernberg, 1941).

SUMMARY

The amounts of bound vitamin B_{12} present
the faeces of one pernicious anemia
atient, three fish tapeworm anemia patients,
ne intestinal megaloblastic anemia patient,
ne carrier of Taenia saginata and a healthy
ubject were examined. The amount of
ound vitamin B_{12} liberated from the ther-
olabile component by boiling varied to
me extent, but the differences observed
ere not great enough to indicate that the
ernicious anemia patient lacking binding
ower had less bound vitamin B_{12} than the
her subjects examined.

REFERENCES

acon, F., Chow, & Yamoto, R.: Properties of B_{12} binding substance in gastric juice. Fed. Proc. *12*, 189, 1953.

eerstecher, E. Jr. & Altgelt, S.: Apoerythein in saliva. J. Biol. Chem. *189*, 31—34, 1951.

erk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R. & Epstein, M.: Observations on the etiologic relationship of achylia gastrica to pernicious anemia. X. Activity of vitamin B_{12} as food (extrinsic) factor. New England J. Med. *293*, 911—913, 1948.

Bethell, F. H., Meyers, M. C. & Neligh, R. B.: Vitamin B_{12} in pernicious anemia and puerperal macrocytic anemia. J. Lab. & Clin. Med. *33*, 1477—1478, 1948.

Castle, W. B., Townsend, W. C. & Heath, C. W.: Observations on etiologic relationship of achylia gastrica to pernicious anemia; nature of reaction between normal human gastric juice and beef muscle leading to clinical improvement and increased blood formation similar to effect of liver feeding. Am. J. M. Sc. *180*, 305—335, 1930.

Diding, N.: A simple cup assay of vitamin B_{12} with a stable requiring mutant of Escherichia coli. Scandinav. J. Clin. & Lab. Investigation *3*, 215—216, 1951.

Hernberg, C. A.: On occurrence of intrinsic factor in gastric juice in pernicious bothriocephalus anaemia. Acta med. Scandinav. Suppl. *123*, 255, 1941.

Kaipainen, W. J. & Tötterman, G.: On the B_{12} vitamin content in faeces in cases of pernicious tapeworm anemia. Scandinav. J. Clin. & Lab. Investigation *6*, 33, 1954.

Siurala, M. & Kaipainen, W. J.: Intestinal megaloblastic anemia treated with aureomycin and terramycin (to be published in Acta. med. Scandinav.).

Ternberg, J. L. & Eakin, R. E.: Erythein and apoerythein and their relation to the antipernicious anemia principle. J. Am. Chem. Soc. *71*, 3858, 1949.

Welch, A. D. & Heinle, R. W.: Hematopoietic agents in macrocytic anemias. Pharm. Rev. *3*, 364, 1951.

ON THE VITAMIN B_{12} CONTENT OF THE FISH TAPEWORM

By W. J. KAIPAINEN and S. WALLÉN

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(Received for publication Nov. 2, 1953)

It has been found (Nyberg, 1952) that *Diphyllobothrium latum,* which may produce a picture typical of pernicious anemia, contains 2.3 μg of vitamin B_{12} per g of dry worm substance. *Ascaris lumbricoides,* which has not been found to cause anemia, contains roughly the same amount of vitamin B_{12}, 1.8 μg per g of dry worm (Nyberg, 1952). The vitamin B_{12} content of the fish tapeworm is independent of whether the worm derives from a host without anemia or from one with anemia induced by the tapeworm (Nyberg, 1952). As regards the cause of tapeworm anemia it has been thought (von Bonsdorff & Gordin, 1952) that the worm consumes large quantities of vitamin B_{12} in the upper part of the intestine producing a picture typical of pernicious anemia. It has ben shown clinically (von Bonsdorff & Gordin, 1952) that a pernicious anemia patient responds well to the administration of dried and pulverized fish tapeworm combined with gastric juice. Dried tapeworm alone gave no positive therapeutic result, which would seem to indicate that the tapeworm does not posses the intrinsic factor. Three out of four cases of pernicious anemia responded to dried tapeworm alone. These three pernicious tapeworm anemia patients evidently had a sufficient amount of the intrinsic factor. It has in fact been demonstrated clinically (Hernberg, 1941) that a response can be obtained by feeding a pernicious anemia patient with the gastric juice of a patient with pernicious tapeworm anemia plus extrinsic factor. In addition, stimulating the gastric juice secretion of a pernicious tapeworm anemia patient with histamine may sometimes produce a blood response.

Ternberg & Eakin (1949) have found substances in gastric juice which bind vitamin B_{12} so as to make it inutilizable by bacteria. They have also found that the gastric juice of pernicious anemia patients contains less vitamin B_{12} binding substance than that of normal subjects. They call the substance apoerythein, and have found it to be thermolabile. In combination with vitamin B_{12} it forms a complex somewhat more thermostabile than apoerythein alone. Boiling liberates bound vitamin B_{12} which can then be determined mirobiologically. Bacon, Chow & Yamamoto (1953) have studied the complex formed by gastric juice and vitamin B_{12} and found that it is soluble in 40 per cent aqueous alcohol but precipitates completely at 60 per cent, and contains more than one fraction by paper chromatography. In addition they have found that the binding power of gastric juice from individuals with pernicious anemia and from old subject is deficient compared with that of healthy adults. Tern-

erg & Eakin (1949) assume that this factor ery probably is the intrinsic factor. As, owever, many substances are capable of nding vitamin B_{12}, Welch & Heinle (1951) oint out that it seems unsafe without good inical evidence to conclude that any binding nenomenon is attributable to the intrinsic ctor. Beerstecher & Altgelt (1951) have und vitamin B_{12}-binding substances in liva also and assume that intrinsic factor ay be involved. However, Castle, Townsd & Heath (1930) have shown that human liva is inert when given to pernicious nemia subjects. On the other hand, Hoffrgensen, Skouby & Andersen (1952) have xtracted from hog pyloric mucosa a thermobile substance with a high vitamin B_{12}nding power. Administering this together ith small amounts of vitamin B_{12} per os ey achievd complete remission in 4 patients ith pernicious anemia, and in one with ypical megaloblastic anemia.

The purpose of the present investigation as to study whether, in addition to the reviously demonstrated free vitamin B_{12}, e fish tapeworm has vitamin B_{12} bound to me thermolabile factor. The fish tapeworm udied derived from persons (a) without nemia, (b) with anemia perniciosa diphyllobothrica, and (c) with anemia perniciosa yptogenetica. *Taenia saginata* was also udied; it is known to contain only 1/50 of e amount of free vitamin B_{12} present in *iphyllobothrium latum* (Nyberg, 1952).

TECHNIQUE

The fish tapeworm was dried and pulvezed. From the powder a suspension was ade containing 1 g of dried and pulverized fish tapeworm and 49 ml of culture medium of the following composition: K_2HPO_4, 7.0 g; KH_2PO_4, 3.0 g; $Na_3 \cdot$ citrate $\cdot 3H_2O$, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $(NH_4)_2SO_4$, 0.1 g; glucose (autoclaved separately), 10.0 g; and water, 1000 g (Diding, 1951).

The suspension was shaken vigorously and filtered in a Chamberlain filter bacteria-free. Free B_{12} was eliminated from the suspension by means of mutant E. coli strain 113—3 which uses vitamin B_{12} in its growth. As the E. coli strain employed required also substances other than vitamin B_{12} for its growth, an addition of culture medium was made as soon as growth ceased, until no growth was obtained even after adding culture medium. The original suspension became so diluted that the amount of dry worm substance was 2.5 mg per ml. The solution was refiltered, boiled for an hour at 100° C, after which it was tested for factors stimulating E. coli strain 113—3, factors which should have been liberated if they were bound with some thermolabile factor. However, this method produced nothing that stimulated the E. coli strain employed. Considerable quantities of vitamin B_{12} have been demonstrated in faeces by the same technique (Kaipainen, Wallén & Tötterman, to be published).

In addition, the investigation was made by boiling at an earlier stage, while the solution still contained free B_{12} vitamin. It was found that the boiling did not increase the growth-stimulating power of E. coli strain 113—3. As 0.00001 μg/ml of vitamin B_{12} is capable of stimulating the growth of E. coli strain 113—3, it seems probable from these experiments that the amount of microbiologically determinable bound vitamin B_{12}

must be less than 0.01 μg of dry worm. With regard to *Taenia saginata* the result was the same as with *Diphyllobothrium latum*.

SUMMARY

An investigation was made as to whether vitamin B_{12}, bound to some thermolabile factor, was to be found in *Diphyllobothrium latum*. Free vitamin B_{12} was removed by employing E. coli strain 113—3 which requires vitamin B_{12} for its growth. An hour's boiling left no substances stimulating the growth of E. coli strain 113—3. Hence it would seem that *Diphyllobothrium latum*, apart from its free vitamin B_{12} content, contains no vitamin B_{12} bound to some thermolabile factor found, *e. g.*, in gastric juice and saliva, at least not in any considerable quantities.

REFERENCES

Bacon, F., Chow, & Yamoto, R.: Properties of B_{12} binding substance in gastric juice. Fed. Proc. *12*, 189, 1953.

Beerstecher, E. Jr. & Altgelt, S.: Apoerythein in saliva. J. Biol. Chem. *189*, 31—34, 1951.

Von Bonsdorff, B. & Gordin, R.: Antianemic activity of dried fish tapeworm. Acta med. Scandinav. Suppl. *266*, 283—292, 1952.

Castle, W. B., Townsend, W. C. & Heath, C. W.: Observations on etiologic relationship of achylia gastrica to pernicious anemia; nature of reaction between normal human gastric juice and beef muscle leading to clinical improvement and increased blood formation similar to effect of liver feeding. Am. J. M. Sc. *180*, 305—35, 1930.

Diding, N.: A simple cup assay of vitamin B_{12} with a stable B_{12} requiring mutant of Escherichia coli. Scandinav. J. Clin. & Lab. Investigation *3*, 215—216, 1951.

Hernberg, C. A.: On occurrence of intrinsic factor in gastric juice in pernicious bothriocephalus anemia. Acta med. Scandinav. Suppl. *123*, 255 1941.

Hoff-Jorgensen, E., Skouby, A. P. & Andersen, J Gad.: Mikrobiologisk standardisering og klinisk effekt af koncentrerede præparater af "intrinsic factor". Nord. med. *51*, 1754, 1952

Kaipanen, W. J. Wallén, S. & Tötterman, G.: On the vitamin B_{12} content in faeces. Scandinav J. Clin. & Lab. Investigation *6*, 85, 1954.

Nyberg, W.: Microbiological investigations on antipernicious anemia factors in the fish tapeworm. Acta med. Scandinav. Suppl. *271*, 1952

Ternberg, J. L. & Eakin, R. E.: Erythein and apoerythein and their relation to the antipernicious anemia principle. J. Am. Chem. Soc *71*, 3858, 1949.

Welch, A. D. & Heinle, R. W.: Hematopoietic agents in macrocytic anemias. Pharm. Rev. *3* 346, 1951.

CINCHOPHEN AND ACETYLSALICYLIC ACID IN ANTICOAGULANT TREATMENT

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Since the discovery of vitamin K it has een realized that a great number of sub- ances influence the clotting time of the lood. This applies primarily to the actual nticoagulants (dicoumarol, indanedione erivatives and others), but to these can be dded drugs whose influence on the clotting me must be termed a more or less essen- al side-effect. Among substances of the tter category, mention may be made of alicylic acid and certain of its derivatives. hey exert the same effect as anti-vitamin (cf. the fact that dicoumarol contains a alicylic group). An indirect effect is xterted by antibiotics administered by outh; they inhibit the bacterial synthesis f vitamin K in the intestine.

It is well established that acetylsalicylic cid and cinchophen can reduce the pro- rombin content of the blood (Link, Over- an, Sullivan, Huebner & Scheel, 1943, apoport, Wing & Guest, 1943, Meyer & oward, 1943, Shapiro, Redish & Camp- ell, 1943, Rawls, 1942, W. C. Hueper, 946), and that hypoprothrombinaemia or ver lesion already present will increase the ffect of salicyl (Shapiro, Redish & Camp- ell, 1943). Further, it has been agreed that ie mechanism is the same as in the case of administration of dicoumarol, only the effect is not nearly so pronounced.

In several recent text-books and surveys on anticoagulant therapy, sections on sali- cylates are often found (a few authors con- sider these to be contraindicated in the course of treatment with dicoumarol), whereas men- tion of cinchophen is looked for in vain (Marble & Wright, 1952, Allen, 1947, Falk, 1947, Duff & Shill, 1949, Shapiro & Weimer, 1949, Sollman, 1948, Phelps, 1950).

The following case record illustrates how much cinchophen is capable of lowering the prothrombin content in the blood when it is low in advance:

A man, aged 66, was admitted to the department with diabetes mellitus and coronary thrombosis. Anticoagulant therapy was instituted. Twelve days later he had an attack of gouty arthritis. In the course of the next 48 hours he was given 4 g of cinchophen in all; this had to be discontinued, as the patient developed nausea and vomiting. On the same day the prothrombin content had fallen to under 5 per cent of the normal. The next morning the patient had hematemeses. The myo- cardium was much impaired in advance, and now he had a hemorrhagic shock. In spite of repeated blood transfusions and treatment with vitamin K, he died 20 hours after the first hematemesis, pre- senting the picture of a pulmonary edema. At autopsy nothing abnormal was found in the ali- mentary canal.

Since cinchophen was not suspected during the treatment as a contributory cause of the hemorrhages, and as only one prothrombin determination was performed in the course of the last 3 days of the patient's life, a number of investigations have been made later to find out whether cinchophen can actually produce such a marked reduction in the prothrombin content.

For the purpose of comparison, similar examinations of the influence of acetylsalicylic acid on low prothrombin concentrations have been performed.

METHODS

The patients examined had been admitted for coronary thrombosis and were being treated with phenylindanedione (dindevan), tromexan or dicoumarol, so that their prothrombin concentration was between 10 and 25 per cent of the normal. All the patients were in a quiescent phase and were closely supervised during the experimental period. No demonstrable complications occurred.

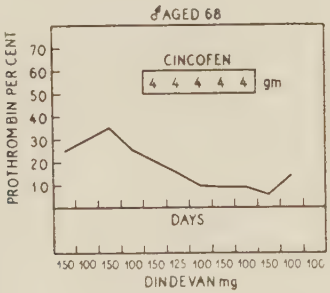
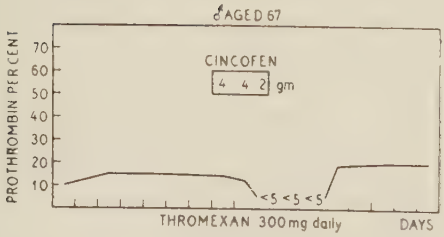


Fig. 1.



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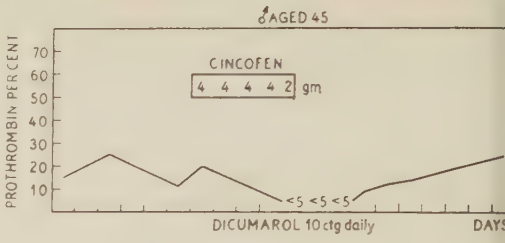

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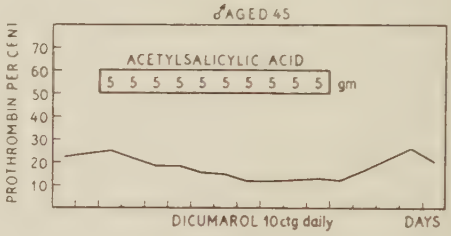

Fig. 4.

The dosage of the actual anticoagulant was kept constant during the entire experimental period in order, as far as possible, to render the variation in the prothrombin content dependent on cinchophen only.

The prothrombin determinations were made according to Owren's method.

RESULTS

The results given in Fig. 1 do not show a very conspicuous effect.

It apppears from Fig. 2 and 3 that the prothrombin concentration falls very much after the administration of cinchophen.

No definite effect of acetylsalicylic acid could be traced in 5 patients (in one, perhaps, a very faint effect). Fig. 4 shows the findings in a patient who was given acetyl-

licylic acid for 10 days. (One week later is patient was given cinchophen; he is the me as is shown in Fig. 3.)

DISCUSSION

The reason why the effect of cinchophen as examined in only 3 patients is that the xamination is not without a certain risk, id that, on the other hand, the effect is gnificant. In one case the fall in the pro-rombin content was but slight (Fig. 1). nfortunately, this patient wanted to be scharged before the experiment had been mpleted.

There are several facts which show that nchophen does not exert a toxic effect on ie liver:

The "latent period" of the cinchophen fect is of the same order of magnitude as iat of dicoumarol. The prothrombin con-nt rises very soon after discontinuance of nchophen. There were no clinical symptoms impaired liver function. Lastly, the same fect of cinchophen has been demonstrated animals fed on a low vitamin K diet (cin-iophen hepatitis is specific in man) (Link al., 1943).

CONCLUSION

Cinchophen must be contraindicated in the urse of anticoagulant treatment, as it may oduce a great fall in the already low pro-rombin concentration.

Drugs containing salicylic acid hardly present any great risk when taken in the doses ordinarily used (*i. e.* from 1 to 3 g daily).

REFERENCES

Allen: The clinical use of dicoumarol, J. A. M. A. *134*, 323, 1947.

Duff & Shill: Fatal hemorrhage in dicoumarol poisoning. J. A. M. A. *139*, 762, 1949.

Falk: Treatment of coronary artery disease. J. A. M. A. *134*, 491,1947.

Hueper, W. C.: Toxicity and detoxication of cinchophen. Arch. of Pathol. *41*, 592, 1946.

Link, K. P., Overman, Sullivan, Huebner & Scheel: Studies on hemorrhagic sweet clover disease; hypoprothrombinemia in rat induced by acetylsalicylic acid. J. Biol. Chem. *147*, 463, 1943.

Marble & Wright: Tromboembolic Conditions. Josiah Macy jr. Foundation (1952).

Meyer & Howard: Production of hypoprothrombinemia and hypocoagulability of blood with salicylates. Proc. Soc. Exper. Biol. & Med. *53*, 51, 1943.

Phelps: The dangers of dicoumarol. The medical clinics of North America, nov. 1950, p. 1791.

Rapoport, S., Wing, M. & Guest, G. M.: Hypoprothrombinemia after salicylate administration in man and rabbits. Proc. Soc. Exper. Biol. & Med., *53*, 40, 1943.

Rawls: Prevention of cinchophen toxicity by use of vitamin K. N. Y. State J. Med. *42*, 2021, 1942.

Shapiro, S., Redish, M. H. & Campbell, H. A.. Studies on prothrombin IV. Proc. Soc. Exper. Biol. & Med. *53*, 251, 1943.

Shapiro & Weimer: Coagulation, thrombosis and dicoumarol (1949).

Sollman: A manual of Pharmacology. (1948.)

THE SEPARATION AND DETECTION OF SERUM ENZYMES BY PAPER ELECTROPHORESIS

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(Received for publication Nov. 11, 1953)

Several studies have been made in the past identifying a number of serum enzymes as components of one or other of the major protein fractions which can be obtained by various procedures.

Vahlquist (1935), using cataphoresis, found that horse serum cholinesterase migrates more slowly than the albumins and faster than the globulins. By fractionation of serum with ethanol at low temperatures, Cohn and his colleagues (Cohn, 1947) found that not all the enzymes studied by them occurred in the same fraction. Carrying further the fractionation with the same technique, Surgenor, Strong, Taylor, Gordon & Gibson (1949) found that cholinesterase was contained in fraction IV-6, which includes part of the α-globulins. By precipitation of a sub-fraction (IV-6-3), Surgenor & Ellis (Goldstein & Doherty, 1951) next showed that the protein with which the enzyme was precipitated was an α_2-globulin.

The close association of cholinesterase with the α-globulins was also demonstrated by Kekwick, McKay & Martin (1953) who used ether precipitation.

Recently, the techniques of paper chromatography and paper electrophoresis have been developed for the purpose of separating enzymes, using small amounts of material. The former method has been used by McKerns (1951) for the separation of casein and rennin. It has also been used in the study of enzymes which can afterwards be identified visually on the paper: thus, Reid (1950) has applied it to the separation of pectin esterase and polygalacturonase of moulds, Giri, Prasad, Gowri Devi & Sri Ram (1952) for amylase, phosphatase and phosphorylase, Gillespie, Jermyn & Wood (1952) for various enzymes of *Aspergillus oryzae*, Jermyn (1952) for horse-radish peroxidase, and Gabib (1952) for phospho glucomutase, invertase, and α-glucosidase.

Following on the earlier work on the major protein fractions of plasma (see Kunkel & Tiselius, 1951), paper electrophoresis has more recently been applied to the separation and identification of several special types of plasma protein; for example, it has been used for the demonstration of lipoprotein (Durrum, Paul & Smith, 1952; Swahn 1952; Fasoli, 1952), of protein-bound carbohydrates (Köiw & Grönwall, 1952) and to identify the protein fractions to which iron (Wallenius, 1952), phosphorus (Maurer & Schild, 1951) and thyroid hormone (Gordon Gross, O'Connor & Pitt-Rivers, 1952) ar

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ound. But so far as we are aware the ethod has not been applied to the study of ood enzymes except by Jacobsson (1953) ho describes the separation of two trypsin hibitors in human serum, although promise f its use for this purpose is provided by the paration and visual demonstration of vege- ble and mould enzymes (Wallenfels & von echman, 1951; Gillespie *et al.*, 1952; ermyn, 1952), of ox-spleen galacturonases Mills & Smith, 1951), and of pancreatic nylase and trypsin (Nikkilä, Ekholm & ilvolà, 1952).

The fact that enzymes are catalytic pro- eins makes possible their identification in ery small amounts through demonstration f the products of enzymic action by some uitable colour reaction. Since we have nown that, after electrophoresis, the various nzymes which we have studied are confined o localized areas on the paper strip, it has een possible to confirm their visual identi- cation by more classical methods, cutting ut appropriate areas of the paper and aking suitable quantitative determinations. The present paper deals with the separa- on and identification by this means of four ydrolytic enzymes normally present in uman serum; ali-esterase, pseudo-cholin- sterase, alkaline phosphatase and amylase.

EXPERIMENTAL

Electrophoretic method

Electrophoretic separation of serum proteins was rried out on paper strips stretched horizontally closed boxes between supports at either end. he paper was Whatman 3 MM and was soaked barbiturate buffer of ionic strength 0.03 *M* and H 8.6. Potential was applied to give 100—150 V ross 26 cm length, but the current was limited 2mA for each 6 cm width of paper.

Serum (0.05—0.2 ml as required) was applied evenly along a 5 cm zone on the wet paper and electrophoresis allowed to proceed for about 16 hr. The paper was then removed and cut longitudinally into 3 strips. One strip was dried at 130° and the proteins on it stained with 1 per cent bromphenol blue in ethanol saturated with mercuric chloride, followed by washing in 0.5 per cent (v/v) acetic acid (Kunkel *et al.*, 1951) and in methanol. The other strips were used for detection of enzymes.

Visual detection of enzymes

(1) *Ali-esterase activity.* The strips were sprayed with tributyrin and incubated in a moist chamber at 37° for about 1 hr. The enzymic liberation of acid was then revealed by spraying either with Universal Indicator (British Drug Houses, Ltd.), diluted to three times its volume with ethanol, or with aqueous cresol red. No attempt was made in these initial experiments to characterize the enzyme or enzymes showing ali-esterase activity (see Discussion).

(2) *Pseudo-cholinesterase.* The same procedure as above was used, except that the substrate was 5 per cent aqueous butyrylcholine perchlorate.

(3) *Alkaline phosphatase.* The method used was evolved from the histochemical procedure of Manheimer & Seligman (1948—9). The strips were sprayed with 0.2 per cent sodium α-naphthyl phosphate in 0.1 *M* carbonate-bicarbonate buffer (pH 9.2), incubated for 1—2 hr., and then developed by spraying with a solution of diazotised *o*-dianisidine, made as follows: 34 mg *o*-dianisidine sulphate were dissolved in 20 ml water and to this were added 20 mg sodium nitrite in 10 ml water, 0.2 ml. 2 *N* H_2OS_4, and then water to make a total of 50 ml.

(4) *Amylase.* The moist paper strip was incubated at 20° for 15—20 hr. on the surface of a plate of agar-starch gel contained in a covered shallow Perspex box. The gel was prepared by dissolving 2 g agar and 1 g soluble starch in 100 ml hot water, and adding 30 ml 0.1 *M* phosphate buffer (pH 7.2). After incubation, the paper was removed and the plate developed by flooding momentarily with aqueous iodine.

Quantitative confirmation of enzymic activity

The reference strip on which proteins had been stained was placed alongside the unused remainder of the paper, which had been kept moist, and bands corresponding to the main protein fractions were marked and cut out; the separated portions were then assayed for enzymic activity by appropriate methods.

(a) *Esterases.* The Warburg technique with bicarbonate buffer (Ammon, 1934) was used, the substrate being either tributyrin (0.03 ml) or 0.03 *M* butyrylcholine perchlorate. The cut portions on the paper bearing the various protein fractions were each shredded and placed in separate flasks. All measurements were carried out in duplicate and corrected for non-enzymic hydrolysis of the substrate; activity is expressed in terms of CO_2 evolved by the protein fraction derived from 1 ml serum.

(b) *Alkaline phosphatase.* The portions of the paper strips were separately incubated at 38° for 30 min. in a mixture of 2 ml carbonate-bicarbonate buffer (pH 9.2) and 0.1 ml 1 per cent sodium α-naphthyl phosphate. After precipitation of protein with 2 ml 10 per cent trichloracetic acid, the tubes were centrifuged; 3 ml of the supernatant were then added to a mixture of 1 ml 2 *N* NaOH with 2 ml ethanol. Diazotised dianisidine solution, 0.1 ml, prepared as described above, was added and the colour read with a Spekker absorptiometer (Spectrum green filter) in comparison with standards made from α-naphthol solution.

(c) *Amylase.* The hydrolysis of starch was followed by estimation of reducing sugar using the method of Haslewood & Strookman (1939). The various portions of paper were incubated (40°, 1 hr.) with 1 ml buffer-substrate made by dissolving 0.75 g soluble starch and 0.2 g sodium chloride in 50 ml 0.1 *M* phosphate buffer of pH 7.2. Proteins were precipitated with 0.2 ml 5 per cent $CuSO_4$ and 0.2 ml 7 per cent sodium tungstate, removed by centrifuging, and 1 ml supernatant used for the estimation of sugar. The colours developed were read against standard glucose solutions.

RESULTS

The electrophoretic procedure used gave sharp resolution of the protein bands, the displacement of the fastest (albumin) fraction being about 17 cm in 16 hr.

Ali-esterase activity was detected as a well defined yellow band on a light green or red ground (according to the indicator used) after incubation at 37° for only 30 min. The yellow patch did not coincide with any of the major protein fractions, but lay between the slowest part of the α_2- and the front of the β-globulins (Fig. 1). In over 20 experiments this relative position was constant.

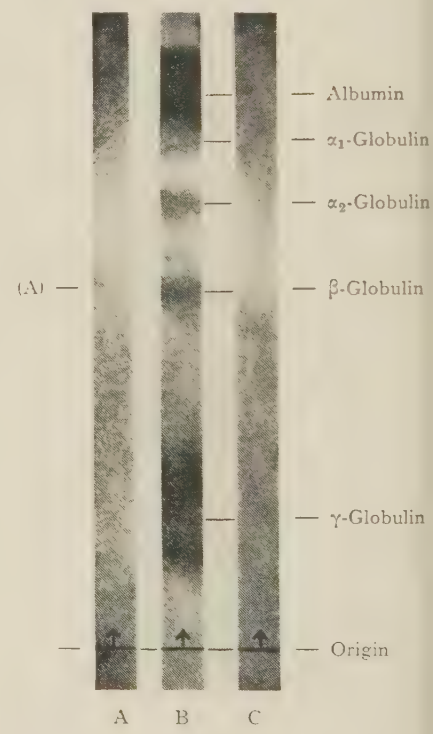

Fig. 1. Cholinesterase (A), proteins (B) and al esterase (C) in serum after electrophoretic separa tion on paper. Esterases revealed by cresol re

No band was revealed when papers bearing e substrate were sprayed with indicator fore incubation. The presence of this zyme between the α_2- and β-globulin peaks as confirmed by the Warburg technique Table I).

a b l e I. *Ali-esterase activity in resolved protein fractions of three normal sera. (Tributyrin as substrate.)*

Fraction	μl CO_2/0.1 ml serum/hr.		
bumin + α_1-globulin	0	9	12
+ β-globulin	218	188	85
globulin	7	18	15

Pseudo-cholinesterase was readily detected the paper strips after incubation for only min. with 5 per cent aqueous butyryl-oline perchloratee. The clearly defined llow patch appeared to be approximately in e same position as that occupied by the ali-terase (Fig. 1). Manometric confirmation ve the results shown in Table II.

a b l e II. *Pseudo-cholinesterase activity in resolved protein fractions of three normal sera.*

(0.03 *M* butyrylcholine perchlorate as substrate.)

Fraction	μl CO_2/ml serum/min.		
bumin + α_1-globulin	4	1.5	2.8
+ β-globulin	73	71	31
globulin	2	1.5	1.5

In preliminary experiments we were un-le to detect alkaline phosphatase on the pers when sodium phenolphthalein phos-ate or phenol phosphate was used as sub-rate. This was so even when sera with thologically raised phosphatase activity re used. Neither spraying of substrate nor e of agar plates containing substrates was

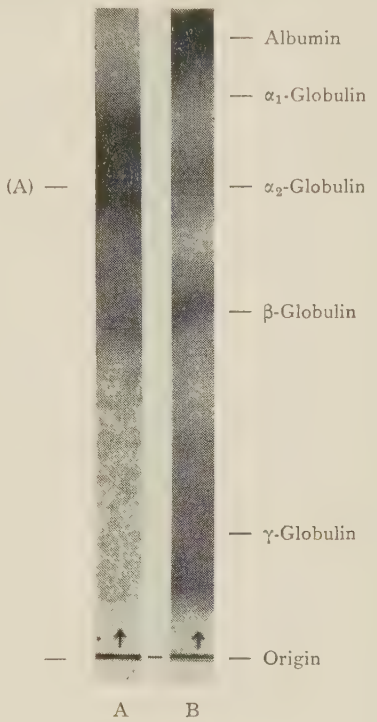

Fig. 2. Alkaline phosphatase (A) and proteins (B) in serum after electrophoretic separation on paper.

sufficiently sensitive. When sodium α-naphthyl phosphate was used as substrate sufficient naphthol was liberated to show up as a purple band on development with diazotised dianisidine. With normal sera the colour indicating enzymic activity was always faint, though clearly perceptible after incubation for 2 hr. The band of colour coincided with the peak of the α_2-globulins. In sera with pathologically raised alkaline phosphatase activity, clearly defined dark coloured bands were revealed after shorter incubation and always occupied the same region (Fig. 2).

The disposition on the paper and the identity of the enzyme were confirmed in subsequent experiments. For the former, por-

Table III. *Alkaline phosphatase activity in resolved protein fractions of three sera.*

Fraction	μg naphthol/ml serum/hr.		
Albumin + α_1-globulin	5.3	6.3	7.9
α_2-globulin	11.0	53.0	21.0
β-globulin	6.1	14.7	10.5
γ-globulin	5.3	11.6	3.2

tions of the paper strip bearing the separated protein fractions were used with the results shown in Table III.

The identity of the phosphatase studied above was established by examination of the pH-activity curve. Using both intact serum and the portion of paper bearing the α_2-globulin fraction, the amount of naphthol liberated in the range pH 8.3—11 in barbitone carbonate buffer (Delory & King, 1939) was determined by the procedure already mentioned, with the results shown in Fig. 3. It will be seen that the curves for whole serum and for the separated enzyme are

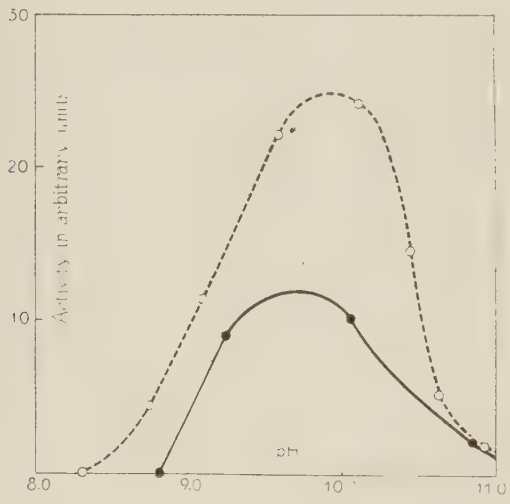

Fig. 3. pH-activity curve for phosphatase in whole serum (- - - - -o- - - - -) and in the α_2-globulin fraction (- —●——).

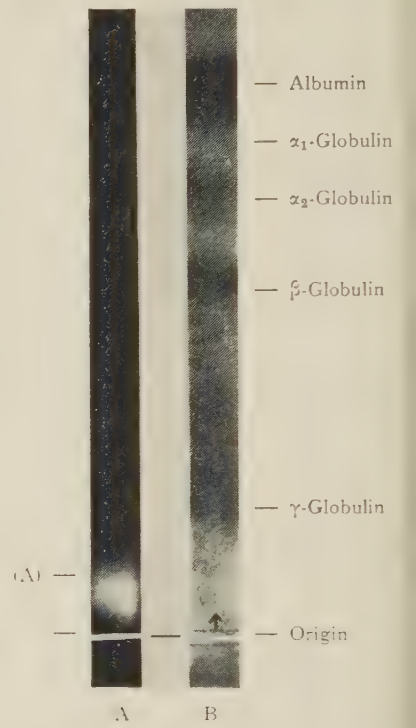

Fig. 4. Amylase (A) and proteins (B) in serum after electrophoretic separation on paper.

similar, and each show a pH-optimum between 9.5 and 10.

Attempts to detect acid phosphatase after electrophoretic separation of normal sera were unsuccessful, even when the above sensitive method was used.

The amylase of the serum resolved on paper strips was best detected by incubation on starch-agar for 15 hr. or more at 20°. Clear indication of the enzyme as a colourless band on the blue ground was always seen with normal sera, and the activity was confined to the slowest part of the γ-globulin in a position where the amount of protein is almost too low to be revealed by bromphenol blue under our conditions (Fig. 4). In on

athological serum in which this stain did ot show the presence of any γ-globulin and which no antibodies had been detected Dent, private communication) a low but stinct amylase activity was still apparent. Confirmation of the identity and location the enzyme were obtained as described ove and gave the results shown in able IV.

able IV. *Production of reducing sugar from starch by resolved protein fractions of four sera.*

Fraction	μg glucose/0.1 ml serum/hr.			
lbumin + α_1-globulin	17	43	25	22
+ + β-globulin	18	14	17	0
globulin (fast portion)	9	0	0	90
globulin(slow portion)	93	190	150	310

DISCUSSION

The results described above show that by paper electrophoretic technique, combined ith suitable methods of detection, it is pos- ble to demonstrate the four serum enzymes hich we have studied as narrow bands aving characteristic mobilities, and standing erefore in definite relationships to the tablished protein fractions. The mobilities ali-esterase and pseudo-cholinesterase pear to be much the same, since both zymes are located between the peaks of the - and β-globulins.Alkaline phosphatase, hich also migrates with the α_2-globulins, incides with the peak of this fraction, and erefore moves a little faster than the two her esterases. Amylase on the other hand haves very differently, and appears to be sociated with the slowest fraction of the globulins. Its mobility therefore differs om that of the amylase in pancreatic juice, which was shown by Nikkilä *et al.* (1952) to migrate much more rapidly. It will be seen from Fig. 2 that in addition to the main zone of alkaline phosphatase activity coinciding with the region of α_2-globulins a second, fainter zone appears to have separated out opposite the β-globulins; a suggestion of this dual localisation of alkaline phosphatase activity has been obtained with several sera, but clearly more work is required before any definite conclusions regarding this can be reached.

As noted above, the hydrolysis of tributyrin and of butyrylcholine under our conditions occurs in fractions having approximately the same mobilities. Although Adams & Whittaker (1949) showed that the cholinesterase of human plasma is responsible for a large part of the tributyrin hydrolysis, they also demonstrated the existence of a separate enzyme hydrolysing this ester. Because of the overlapping substrate specificity of many of the esterases it is not easy to interpret the hydrolysis of tributyrin which has been demonstrated in terms of the particular enzyme or enzymes involved. We have not, however, obtained any evidence by our visual procedure of a second tributyrin-splitting enzyme possessing any great difference of mobility, so that it would seem that the ali-esterase which splits this substrate must move at approximately the same rate as the pseudo-cholinesterase. Vahlquist (1935) has also shown that the acetylcholine and tributyrin-esterase activities of human plasma are not separated by cataphoresis.

Although this electrophoretic technique does not seem to offer any appreciable advantage over the methods generally in use for the estimation of these enzymes, it offers

the possibility of investigating the mobilities in addition to the activities of enzymes in conditions of disease.

In interpreting the results obtained with this technique it is of course, necessary to bear in mind the limitations of paper electrophoresis, in which the final distribution of proteins depends not only on the action of the electric field, but also on movements of the buffer as well as on ill-defined physicochemical effects occurring within the material under treatment.

SUMMARY

1. Methods are described for the visual detection of four hydrolytic enzymes in normal human serum after electrophoretic separation on paper.
2. Alkaline phosphatase was found to migrate with the peak of the α_2-globulins, pseudo-cholinesterase and aliesterase activity were found between the α_2- and β-globulin peaks, and amylase in the slowest-moving part of the γ-globulins.
3. The identities and distributions on the papers of these enzymes have been confirmed by quantitative methods.

ACKNOWLEDGEMENTS

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REFERENCES

Adams, D. H. & Whittaker, V. P.: The charac terisation of the esterases of human plasma Biochem. J. *44*, 62, 1949.

Ammon, R.: Die fermentative Spaltung des Acetyl cholins. Pflüg. Arch. ges. Physiol. *233*, 486 1934.

Cabib, E.: Paper chromatography of some en zymes and the plasma proteins. Biochem. Bio phys. Acta. *8*, 607, 1952.

Cohn, E. J.: Chemical, physiological and immuno logical properties and clinical uses of bloo derivates. Experientia. *3*, (4), 125, 1947.

Delory, G. E. & King, E. J.: The rates of en zymic hydrolysis of phosphoric esters. Biochem J. *33*, 1185, 1939.

Durrum, E. L., Paul, M. H. & Smith, E. R. B. Lipid detection in paper electrophoresis. Science *116*, 428, 1952.

Fasoli, A.: Electrophoresis of serum lipoprotei on filter paper. Lancet, I, 106, 1952.

Franklin, A. E. & Quastel, J. H.: Paper chromat graphy of protein mixtures and blood plasm Proc. Soc. exper. Biol. Med. *74*, 803, 1950.

Gillespie, J. M., Jermyn, M. A. & Woods, E. F. Multiple nature of the enzymes of *Aspergillu oryzae* and of horse-radish. Nature *169*, 487 1952.

Giri, K. V., Prasad, A. L. N., Gowri Devi, S. & Sri Ram, J.: A technique for the identificati and separation of enzymes by paper chromat graphy. Biochem. J. *51*, 123, 1952.

Goldstein, A. & Doherty, M. E.: Stability a thermal denaturation of purified human plasma cholinesterase. Arch. Biochem. Biophys. *33* 22, 1951.

Gordon, A. H., Gross, J., O'Connor, D. & Pitt Rivers, R.: Nature of the circulating thyroi hormone-plasma protein complex. Nature *169* 19, 1952.

Haslewood, G. A. D. & Strookman, T. A., A metho for the estimation of "true" sugar in 0.05 m of blood. Biochem. J. *33*, 920, 1939.

Jacobsson, K.: Electrophoretic demonstration o two trypsin inhibitors in human blood serum Scandinav. J. Clin. Lab. Investigation *5*, 97 1953.

rmyn, M. A.: Horse radish peroxidase. Nature *169,* 488, 1952.

nes, J. I. M. & Michael, S. E.: Chromatography of proteins. Nature *165,* 685, 1950.

ekwich, R. G. O., MacKay, M. E. & Martin, N. H.: The preliminary isolation and characterisation of human serum cholinesterase. Biochem. J. *53,* xxxvi, 1953.

öiw, E. & Grönwall, A.: Staining of protein-bound carbohydrates after electrophoresis of serum of filter-paper. Scandinav. J. Clin. Lab. Investigation *4,* 244, 1952.

unkel, H. G. & Tiselius, A.: Electrophoresis of proteins on filter paper. J. gen. Physiol. *35,* 89, 1951.

cKerns, K. W.: Paper chromatography of casein and rennin. Canad. J. Med. Science *29,* 59, 1951.

anheimer, L. H. & Seligman, A. M.: Improvement in the method for the histochemical demonstration of alkaline phosphatase and its use in a study of normal and neoplastic tissues. J. Nat. Cancer Inst. *9,* 181, 1948—9.

laurer, W. & Schild, K. T.: Papier electrophorese der phosphorhaltigen Serum bestandteile. Klin. Wchnschr. *29,* 514, 1951.

ills, G. T. & Smith, E. E. B.: The electrophoresis of enzymes on filter paper. Biochem. J. *49,* vi, 1951.

ikkilä, E., Ekholm, K. & Silvolà H.: Paper electrophoresis of enzymes; amylase and trypsin. Acta chem. Scandinav. *6,* 617, 1952.

Reid, W. W.: Estimation and separation of the peptin-esterase and polygalacturonase of microfungi. Nature *166,* 569, 1950.

Surgenor, D. M., Strong, L. E., Taylor, H. L., Gordon, R. S. & Gibson, D. M.: The separation of choline esterase, mucoprotein and metal-combining protein. J. Am. chem. Soc. *71,* 1223, 1949.

Swahn, B.: A method for localisation and determination of serum lipids after electrophoretical separation on filter paper. Scandinav. J. Clin. Lab. Investigation *4,* 98, 1952.

Vahlquist, B.: On the esterase activity of human blood plasma. Skand. Arch. Physiol. *72,* 133, 1935.

Wallenfels, K. & v. Pechmann, E.: Über die trennung v. Enzymgemischen durch Electrophorese in Filtrierpapier. Angew. Chem. *63,* 44, 1951.

Wallenius, G.: A note on serum iron transportation. Scandinav. J. Clin. Lab. Investigation *4,* 24, 1952.

NOTE. Since the completion of this work a short account by A. Delcourt & R. Delcourt (1953, C. r. Soc. Biol., *147,* 1104) has appeared describing the electrophoretic separation of esterases and amylase in mouse-serum. They refer to a few experiments with human serum, in which, although they identified esterase activity, they were only able to demonstrate phosphatase in cases where the enzyme was pathologically increased.

THE EFFECT OF LONG-ACTING INFILTRATION ANESTHESIA WITH EFOCAINE ON THE RESPIRATORY MOVEMENTS AT REST POSTOPERATIVELY

A PHOTOGRAMMETRIC STUDY

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Impaired respiration is considered to play a major etiologic role in postoperative atelectasis.

Such an impairment demonstrated by Muller, Overholt & Pendergrass (1929), who recorded the diaphragmatic movements under fluoroscopy pre- and postoperatively with the patients recumbent and in resting respiration. The postoperative magnitude of the respiratory movements was 55 per cent of the preoperative values.

The impaired respiration has been attributed to pain from the wound, among other factors.

Gius (1941) successfully treated two cases of postoperative atelectasis by intercostal nerve block. Zolliger (1941), in order to obtain a more prolonged effect, used eucupine and procaine in oil as an infiltration anesthesia in abdominal operations. He found a greater vital capacity and fewer pulmonary complications than in the control series. Starr & Gillman (1942) used nupercaine in oil for intercostal nerve block following abdominal operations. They, too, studied the vital capacity postoperatively, and found it to be greater than that in the control series.

Pooler (1949) recommended breathing exercises as a prophylaxis against postoperative atelectasis, and before the patient commenced the exercises procaine was given intravenously as an anesthetic.

Blades & Ford (1950), when performing intrathoracic operations, used polyethylene tubes which were inserted in the thoracic wall at the conclusion of the operation. Procaine was then instilled through the tubes postoperatively. Gervig, Thompson & Blades (1951) employed the same technic in abdominal operations. In midline incisions above the umbilicus, three tubes were inserted in front of the rectus fascia, one of them at the upper end, one at the lower end, and a third in the middle of the wound. The number of morphine injections required was taken as a criterion of the degree of pain. Those patients who received local anesthesia needed only one-third as many morphine injections postoperatively as did the controls. Following the demonstration by Judowitch & Bates (1949) that ammonium ions have an anesthetic effect, Davis & Wilson (1950) sought to produce infiltration anesthesia with ammonium sulphate at abdominal operations. The patients were examined daily and questioned as to the degree of pain. Expressing the effect of anesthesia as grade 1, 2 or 3, the authors found that ammonium

lphate gave subjective relief of pain for e days.

In 1946, Rappaport described durocaine, hich consists of benzocaine dissolved in per cent urethane solution. On injection e benzocaine is precipitated and forms a pot of benzocaine crystals at the injec- n site.

To secure an objective expression of ocking of the origin of the pain, Hagberg 952) studied photometrically the post- erative pallor reflex which Adams-Ray 944, 1949, 1950) had shown to be a con- ant symptom of the inflammatory syn- ome. After infiltration anesthesia had en produced with durocaine in every cond case of twenty cholecystectomies, the stoperative pallor reflex was abolished in ne of the ten anesthetized cases. More- er, there was an appreciable subjective nelioration of pain from the wound in the nesthetized patients, as compared with the ntrols.

Efocaine, constituted on the same prin- ples as durocaine, is a solution of procaine gether with propylene and polyethylene ycol. An experimental investigation con- ucted by Weinberg (1952) on rabbits, inea pigs and rats showed that administra- n of efocaine was not associated with any ermanent damage to the tissues. Nor was ny foreign-body reaction demonstrable. focaine has been used as a postoperative ialgesic in upper abdominal operations by son & Shaftel (1952). They obtained nesthesia with a duration of 8—14 days. The atients did not require morphine to the me extent as the controls.

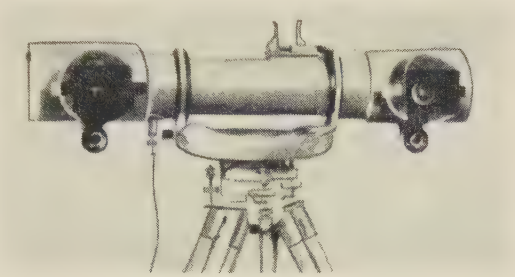

Fig. 1.

METHOD

For the purpose of measuring the respiration in connection with operative measures, the vital capacity has earlier been determined. This method provides a measure of the maximum respiratory effort.

We felt that it would be of interest to investigate the resting respiration in some patients under long-acting infiltration anesthesia and in others without anesthesia.

For this purpose we employed a photogrammetric procedure. One black sheet was fixed on the lower thorax and another on the upper abdomen; and to them were gummed small glossy pellets. An exposure of one minute was then made with a stereocamera (Fig. 1) placed at a constant distance (140 cm) from the patient. On the photographic plates the pellets formed lines. By measuring these lines a value for the mean respiration over a period of one minute could be calculated. Measurement was done with the aid of a microscope fixed to a measuring instrument, with a mean error of 2 μ. By deducting the pellet's diameter from the distance measured, a value was obtained for the movement of the pellet.

In twelve of twenty cases of cholecystectomy performed *via* an oblique incision below the right costal arch, infiltration anesthesia was induced with 30 cc efocaine. The magnitude of the respiratory movements was determined preoperatively and on the third day after operation.

Further, the occurrence of a postoperative pallor reflex was investigated photometrically on the third day after operation. In the anesthetized cases this test was made in a *non-anesthetic area* in the neighbourhood of the wound.

Fig. 2.

RESULTS

The ratio between the difference in the preoperative and postoperative respiration on the one hand, and the preoperative respiration on the one hand, and the preoperative respiration on the other, was calculated in respect of both thoracic and abdominal breathing in each patient, and the mean was determined.

X = Mean of $\frac{\text{difference in pre- and postoperative values}}{\text{preoperative values}}$ in respect of thoracic breathin in the anesthetized patients.

X^1 = Mean of $\frac{\text{difference in pre- and postoperative values}}{\text{preoperative values}}$ in respect of thoracic breath ing in the non-anesthetize patients.

Y = Mean of $\frac{\text{difference in pre- and postoperative values}}{\text{preoperative values}}$ in respect of abdominal breathing in the anesthetize patients.

Y^1 = Mean of $\frac{\text{difference in pre- and postoperative values}}{\text{preoperative values}}$ in respect of abdominal breathing in the non-anesthe tized patients.

$$X = 0.35 \quad X^1 = 0.35 \quad Y = 0.47 \quad Y^1 = 0.27$$

Analysis by Student's test shows no statistically significant difference between the groups. If the postoperative respiration is expressed in per cent of the preoperative, a reduction to approximately 60 per cent in the resting will be obtained in all groups (Fig. 2).

In no anesthetized case did any postoperative pallor reflex occur. This reflex wa demonstrated, on the other hand, in a controls.

COMMENTS

Subjectively, the anesthetized patient experienced only very slight postoperativ pain from the wound. The absence of th postoperative pallor reflex shows that th

igger point had been blocked. This pro-
des objective evidence that they felt less
ronounced postoperative pain from the
ound than did the controls. This notwith-
anding, the anesthetized patients showed
reduction of the resting respiration to the
me degree as the controls. Probably, there-
re, pain from the wound is of no signi-
cance relative to the magnitude of the re-
iration *at rest*. The patients were operated
with the same procedure and, mostly, by
e same surgeon. The postoperative reduc-
on of the respiratory movements at rest
ay conceivably be due, in part, to impaired
asticity of the abdominal wall, brought
out by the operation and the postoperative
velling in the wound. The value obtained
r the postoperative respiratory reduction
closely consistent with the results reported
y Muller, Overholt & Pendergrass (1929)
ith regard to the postoperative diaphrag-
atic movements. The increase of the vital
apacity which Zolliger (1941) and Starr &
illman (1942) found in postoperative use
local anesthetics suggests that this form
analgesia is of great benefit in bringing
out *excessive respiratory movements*. Post-
peratively, breathing exercises are of con-
derable help in preventing atelectasis, and
ne of their aims is, of course, to obtain the
reatest possible respiratory movements.
resumably the magnitude of respiration at
st is not of the same fundamental impor-
nce in this connection.

SUMMARY

A photogrammetric procedure for de-
rmining the thoracic and abdominal respira-
ry movements is presented. Of twenty
atients who had undergone cholecystectomy,
twelve received infiltration anesthesia with
efocaine. The anesthetized patients had only
very slight subjective pain from the wound.
The occurrence of a postoperative pallor
reflex was tested photometrically. The reflex
was demonstrated in all controls but in none
of those who had received anesthesia. No
statistically significant difference with regard
to the resting respiration was found between
the anesthetized patients and the controls.
The magnitude of the respiratory movements
on the third day after operation was
approximealty 60 per cent of the preopera-
tive value.

REFERENCES

Adams-Ray, J.: Edema, hyperalgesia and Paleness, Uppsala, Almquist & Wiksells Boktryckeri AB, 1944.

— &Hagberg, S.: L'Hypertonie des capillaires veineux. Nouvel élément du syndrome inflammatoire. Lyon Chir. *6*, 933, 1949.

— Pallor — ein kutaner Sympaticusreflex im Inflammationssyndrom — Speziell nach chirurgischen Eingriffen im oberen Teil des Bauches. Zentr.bl. f. Neurochir. *1*, 3, 1950.

Blades, B. & Ford, W.: Method for control of postoperative pain. Surg., Gynec. & Obst. *91*, 524, 1950.

Davis, H. & Wilson, C.: Ammonium sulfate as a long-acting analgesic. Am. J. Surg. *81*, 318, 1951.

Gervig Jr., W. Thompson, C. & Blades, B.: Pain control following upper abdominal operations. Arch. Surg. *62*, 678, 1951.

Gius, J. A.: Paravertebral procaine block in the treatment of post-operative atelectasis Surgery *8*, 832, 1940.

Hagberg, S.: Postoperative Infiltration Anesthesia in Cholecystectomy and its Effect on Cutaneous Vasomotor Reflexes. Acta chir. Scandinav. *104*, 329, 1952.

Iason, A. & Shaftel, H.: A New Approach to the Problem of Postoperative Pain. Am. J. Surg. *83*, 549, 1952.

Judovitch, B. & Bates, W.: Pain Syndromes, III. Edition, Philadelphia, Davis *242*, 1949.

Muller, G., Overholt, R. & Pendergrass, E.: Postoperative Pulmonary Hypoventilation. Arch. Surg. *19*, 1322, 1929.

Pooler, H.: Relief of postoperative pain and its influence on vital capacity. Brit. Med. J. *2*, 1200, 1949.

Puderbach, W. & Shaftel, H.: Prolonged Intercostal Nerve Block in Upper Abdominal Surgery. Lancet, *72*, 203, 1952.

Rappaport, F.: An anesthetic with prolonged actior Acta med. Orient. *5*, 4, 1946.

Starr, A. & Gillman, S.: The effect of post operative intercostal nerve block with nuper cain oil following upper abdominal surgery New England J. M. *227*, 102, 1942.

Weinberg, T.: A Study of the Effects of Efocain upon Nerves, Skin and Subcutaneous Tissue Fougera Scint. Bull., 2. Jan. 1952.

Zollinger, R.: Observation on uses of prolonge anesthetic agents in upper abdominal opera tions. Surgery *10*, *27*, 1941.

THE URINARY EXCRETION OF GLUTAMINE AND GLUTAMIC ACID IN NORMALS AND IN A GROUP OF PSYCHIATRIC PATIENTS

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(Received for publication November 25, 1953)

Munkvad (1951) has shown that the concentration of glutamic acid in blood plasma is lower in patients belonging to the group of so-called schizophrenia than in a group of normal control subjects. Further investigations of the metabolism of glutamine and glutamic acid therefore, perhaps, will be of some psychiatric interest in the future.

In order to investigate the urinary excretion of glutamine and glutamic acid, a new method for the quantitative determination of these two substances in urine has been worked out by the author. This method and its application on two normal males has been described previously in this paper (1953). By means of this method the daily urinary

Table I. *The daily urinary excretion of glutamine and glutamic acid in 7 normal males.*

ame	Age	Body weight	Date	Diuresis	pH of urine	Specific weight of urine	Glutamine in mg	Glutamic acid. in mg
. R.	29 years	74 kg	July 19, 1953	1070	5.6	1026	42	5
			July 22, 1953	1800	6.0	1018	55	4
			July 23, 1953	1530	5.8	1022	49	4
. O.	58 years	86 kg	Sept. 7, 1953	1430	5.0	1012	66	7
			Sept. 8, 1953	1060	5.0	1020	46	8
			Sept. 10, 1953	1010	5.2	1020	49	8
. C.	53 years	80 kg	Sept. 9, 1953	860	6.0	1028	103	4
			Sept. 10, 1953	1010	5.6	1020	88	5
			Sept. 11, 1953	1270	5.6	1018	89	6
. B.	34 years	88 kg	Sept. 10, 1953	890	5.8	1032	114	9
			Sept. 11, 1953	1010	5.6	1030	98	10
			Sept. 12, 1953	1670	6.0	1021	116	12
. B.	40 years	72 kg	Sept. 16, 1953	890	6.0	1020	47	4
			Sept. 17, 1953	890	6.0	1020	43	4
			Sept. 18, 1953	900	6.4	1022	59	2
. L.	33 years	75 kg	Sept. 16. 1953	1560	6.4	1020	174	7
			Sept. 17, 1953	1020	6.4	1026	175	5
			Sept. 18, 1953	1350	6.2	1024	163	7
G.	48 years	58 kg	Sept. 22, 1953	730	5.8	1028	54	3
			Sept. 23, 1953	760	6.0	1025	57	3
			Sept. 24. 1953	1070	5.8	1020	75	5

Table II. *The daily urinary excretion of glutamine and glutamic acid in 9 male patients belonging to the group of so-called schizophrenia.*

Name	Age	Body weight	Date	Diuresis ml	pH of urine	Specific weight of urine	Glutamine in mg	Glutamic acid in mg	Remarks
H. R.	48 years	67 kg	July 20, 1953	1640	6.2	1016	40	5	Predominantly paranoid-hallucinatoric psychosis with some catatonic trends, deteriorated, authistic. Mixed leptosome-pycnic type. Predominantly schizoid.
			July 21, 1953	1650	6.5	1014	34	5	
			July 22, 1953	1120	6.2	1022	47	4	
H. T.	50 years	70 kg	July 20, 1953	1000	5.6	1024	70	5	A series of psychotic phases with catatonic-paranoid symptomatology during the last 20 years in a leptosome-athletic mixed type. Schizoid, with cyclic trends.
			July 21, 1953	1130	6.0	1020	57	4	
			July 22, 1953	1200	6.0	1022	67	6	
J. R.	39 years	70 kg	July 22, 1953	1140	6.4	1022	88	4	Insidious, chronic elapsing process-psychosis of 10 years duration with autistic, paranoid-catatonic symptoms. Predominantly leptosome type.
			July 23, 1953	1530	6.4	1020	80	6	
			July 24, 1953	970	6.2	1027	77	5	
J. S.	36 years	66 kg	July 22, 1953	680	5.8	1028	64	4	Chronic, insidiously elapsing, slowly deteriorating process-psychosis of 17 years duration in an athlethic-leptosome mixed type. Catatonic trends.
			July 23, 1953	720	6.0	1026	45	4	
			July 24, 1953	470	5.6	1032	50	2	
E. G.	46 years	80 kg	Sept. 6, 1953	930	6.6	1022	58	8	Phasic to periodic catatonic state — completely asyntone — of 26 years duration in an extremely leptosome type.
			Sept. 7, 1953	1075	6.5	1022	78	6	
			Sept. 8, 1953	610	6,8	1027	59	5	
K. C.	28 years	65 kg	Sept. 11, 1953	820	6.6	1020	46	2	A mixed catatonic-paranoid-hallucinatoric state since 1948. Traumata capitis 5 and 12 years old. Involuntary movements of left side. Previously trends to chronic encephalitic changes in the spinal fluid. Leptosome.
			Sept. 12, 1953	790	6.6	1020	40	2	
			Sept. 13, 1953	830	6.2	1022	49	2	
O. B.	40 years	72 kg	Sept. 11, 1953	720	7.4	1026	40	3	Slowly progressing, mostly endogenous process-psychosis of catatonic symptomatology in a schizoid, intellectually gifted leptosome-athletic mixed type. 11 years duration.
			Sept. 12, 1953	890	6.5	1022	41	4	
			Sept. 13, 1953	780	6.0	1024	40	2	
O. G.	29 years	60 kg	Sept. 11, 1953	1030	6.4	1026	68	8	Post-traumatic, depressive-catatonic state since 1946. Treated in two mental hospitals previously. Leucotomia 1947. Now dull, demented. Dysplastic type. More cases of psychosis among near relatives.
			Sept. 12, 1953	1330	6.4	1018	68	6	
			Sept. 13, 1953	810	5.8	1024	82	11	
F. B.	31 years	77 kg	Sept. 15, 1953	910	5.6	1025	101	5	Subacute attack of catatonic-paranoid psychosis of 2 years duration. Some misuse of alcohol the last 6—7 years. Insulin and E. C. T.-treatment
			Sept. 16, 1953	940	5.6	1018	88	5	
			Sept. 17, 1953	990	5,8	1020	84	2	

cretion of glutamine and glutamic acid
s been followed up for three days in 7
rmal males and in 9 patients belonging
the group of so-called schizophrenia. The
tients examined are male subjects mostly
presenting the group of chronics. Before
e examination was started every patient
s thoroughly physically examined. Gener-
y the patient's body-temperature, pulse
e and body weight were registered during
ong period without notable changes. The
dinary examination of organs and organ-
nctions showed no pathological findings
d their general physical state at the time
examination was good. The normal con-
l subjects too were healthy and in good
ysical condition. All persons examined
re on an ordinary diet except two patients
o were given a diet consisting of a mixture
milk, cream, egg, sugar, the necessary
neral salts and vitamins, containing suffi-
nt amounts of proteins and calories. The
sults are shown in the following tables.

SUMMARY

The urinary excretion of glutamine and glutamic acid in a group of psychiatric patients and in a group of normal control subjects has ben determined. The excretion of glutamine shows considerable individual variations, from about 35 mg to about 175 mg *pro die*. The excretion of glutamic acid, however, seems to be more constant. It was found to range between 2 and 12 mg *pro die*. In none of the patients did the amounts of glutamine and glutamic acid excreted differ from the amounts found in the group of normal control subjects.

REFERENCES

Astrup, P. & Munkvad, I.: Glutamin- og glutaminsyrestoffskiftet. With an English summary. Nord. med. *45*, 117, 1951.

Munkvad, I.: Glutaminsyre og glutaminbestemmelser i plasma på et psykiatrisk patientmateriale. (Disputas.) With an English summary. Copenhagen 1951.

Foss, O. P.: On the determination of glutamine and glutamic acid in urine by a modification of Krebs' method. Scandinav. J. Clin. & Lab. Investigation *5*, 334, 1953.

ON THE SIGNIFICANCE OF THE BIPOLAR CHEST LEAD IN ECG BETWEEN APEX CORDIS AND THE LEF POSTERIOR AXILLARY LINE

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In clinical electrocardiography both standard limb leads and unipolar chest leads are used as a routine. The limits of the normal electrocardiogram are drawn from many investigations. The range of variation is large, however, and the values for the chest leads are given in absolute figures (Grewin 1948, Lepeschkin 1951).

A chest electrode records potential variations from a limited area of the cardiac surface only, as emphasized by Lewis (1909—10). If a myocardial affection is slight, or localized to the outer part of the projection of the electrode, small or no changes in the ECG can be expected on the surface. The absolute value of the ECG in the lead may still be within normal limits, but in relation to other leads more distant from the process, deviations from the normal might be found. A comparison of directly measured values in ECG in different chest leads should reveal such relative changes, but in routine work the shortage of time makes such a procedure impracticable. With the aid of bipolar chest recording, the difference between the ECG from two chest points is obtained directly and can be evaluated easily.

Bipolar leads were used early (Drury & Iliescu 1921, Trendelenburg 1934, Nylin & Sällström 1938, Nehb 1938 and others) but have been abandoned more and more, favour of the unipolar leads. The standardiz tion of ECG has been of extreme importan but a disadvantage has been that oth methods of recording ECG have perha been pushed into the background.

The purpose of the present investigati has been to study the value of a bipolar che lead between the apex and position C_7 in clinical material. This lead called J, accordi to the nomenclature of Nehb, has be studied by Bengtsson (1947), Grewin (194 and Carlsten (1952) and has been found be of certain importance. In the diagnos of myocardial diseases the T-wave is of prin interest. Normally the T-wave is usually higher amplitude over the apical region tha at more lateral chest positions (Roche 194 Hill 1946) and consequently the T-wave lead J is more or less positive, as this le constitutes the difference of the ECG fro the apical region and position C_7 in t posterior left axillary line. Carlsten (195 studied 33 cases with normal standard lim leads and unipolar chest leads but with neg tive T-waves in lead J. The results indicat a possibility of differentiating between path logical and physiological changes of the wave in J depending upon the depth of neg tivity of this wave. The present paper dea

ith an extension of this investigation. 116 ases showing normal ECG, in standard limb nd unipolar chest leads but with negative -waves in J have been analyzed from a inical point of view. It was hoped to try establish whether this pattern of ECG was nsistent with a normal myocardium or not.

METHODS

An ordinary amplifier electrocardiograph (Clinic, lema) was used. All cases were examined with e standard limb leads and the following chest ads, CR_7, IV R, CR_2 and the bipolar lead J, *i. e.* e recording between apex and C_7. In addition ther CR_1 or CR_5 was as a rule employed.

The amplitude of the T-wave was measured from line drawn through the middle of two consecutive -R segments to the point of the T-wave, where e amplitude was greatest. For a value of the gativity of the T-wave, the mean of the values three separate T-waves was taken and expressed tenths of a millimeter. A special control of the nplification of the two leads IV R and CR_7 was cessary and corrections were made for differences voltage.

The determination of the physical working capaty was made according to the routine method ed in the laboratory and described by Sjöstrand 947, 1950) and Wahlund (1948). Chest lead ECG as recorded during each load and immediately and minutes after the end of the work. The hypomia test was performed with 9 per cent oxygen nitrogen, during 8 minutes.

To estimate if the physical work performed was thin normal limits, the total amount of hemobin was calculated in some of the patients acrding to the method described by Sjöstrand (1948) d the heart volume according to Kjellberg, Rudhe Sjöstrand (1949). The same authors found a gh linear correlation between the physical workg capacity expressed as the work load at a pulse te of 170 a minute on the one hand, and the heart lume and the amount of hemoglobin on the ner.

Ordinary clinical data were considered in evalung the diagnosis. A special follow-up investigan was made in doubtful cases.

MATERIAL

Patients of both sexes are included in the material. The age range is from 15 to 72 years. The material is selected according to criteria described above, *i. e.* standard limb leads and CR leads within normal limits, as described by Lepeschkin (1951) and with negative T-waves in lead J. A lower T-wave in IV R compared with CR_7 can be due to (i) decreased amplitude of the T-wave in CR_2 which automatically reduces the T-wave in IV R and (ii) a localized myocardial affection of the apical area only, interfering with the potential variations in ECG from the precordium over apex cordis. Only cases belonging to the last mentioned group are included in the material and in all, 116 cases were examined.

The material was divided into five groups.

I. *Normal working capacity and normal ECG during and after work, or normal hypoxemia reaction.*

♂ 18 cases ♀ 7 cases

II. *Series of ECG and the other clinical data indicating a normal myocardium.*

♂ 2 cases ♀ 13 cases

III. *Low physical working capacity.*

♂ 5 cases ♀ 1 case

IV. *Subjective and electrocardiographic signs of coronary insufficiency or myocardial affection during or after work, or hypoxemia test.*

♂ 14 cases ♀ 15 cases

V. *Series of ECG and the other clinical data indicating myocardial disease.*

♂ 21 cases ♀ 20 cases

Total ♂ 60 cases. ♀ 56 cases.

In group I three cases were examined with the hypoxemia test, in group IV eight cases.

RESULTS

Groups I and II containing the cases judged as normal, consist of 20 female and 20 male cases. The negative phase of the T-wave in lead J is found to be 0.38 mm, SD: 0.18 for female and 0.30 mm, SD: 0.20 for male subjects.

In groups III, IV and V consisting of pathological cases, the negativity of the T-wave in the bipolar recording is more pronounced. In female patients the negativity was 0.94 mm, SD: 0.51 and in male patients 1.26 mm, SD: 0.79.

Thus there seems to exist a possibility of separating definitely pathological cases on the one hand and normal and doubtful cases on the other. Negativity of the T-wave in J of about one mm or more is evidence of a myocardial affection. If the negativity is less pronounced the case might be normal.

In the present material several examples of the pathological significance of a negative T-wave in J were found. A slightly negative phase of the T-wave in J, as the first sign of a myocardial process, is seen in Fig. 1.

Case history (Fig. 1). A 44 year old labourer. Previously healthy. 1949 slight precordial pain on effort, radiating to the right shoulder and arm. No subjective or objective signs of heart failure. SR: 10 mm/hour.

In May 1951 had for two weeks the same symptoms as two years before. In bed for three weeks. Then started his work again but in July of the same year more attacks of precordial pain, now most often when emotionally agitated. Began his work again in September of the same year. His symptoms were unchanged until March 28 and 31, 1953 when sever pains forced him to hospital. SR: 33 mm/hour. The blood pressure during 1949—1953 was systolic 165—140 and diastolic 110—90 mm Hg.

Clinical diagnosis: infarction.

Case history (Fig. 2). A 27 year old nurse. 1940 rheumatoid arthritis. In bed for one year. No ECG changes at that time. 1951 and 1953 symptome of lumbago. Slight palpitations. SR: Febr. 13 and 18, 1953 9 mm/hour. Antistreptolysin titre: 800 U in repeated samples.

Clinical diagnosis: myocarditis.

As a negative phase of the T-wave in lead J might be the first sign of a pathological process in the myocardium, so it might also be the last change to disappear (Fig. 3).

Case history (Fig. 3). A 52 year old fisherman. Previously healthy. Since autumn 1950 two attacks on effort of precordial pain radiating to the neck and the jaw. During the winter of 1951 several attacks on effort during cold weather. January 1952 increasing intensity of attacks. Admitted to the hospital February 14, 1952. SR: 2 mm/hour. Blood pressure 175—135 systolic and 100—85 diastolic during the stay in the hospital (Febr 2—March 24, 1952).

Clinical diagnosis: infarction.

The following example illustrates the importance of a work test in cases with negative T-wave in J.

Case history (Fig. 4). A 57 year old housewife. During the last 6 months diffuse precordial pains on effort. Blood pressure 170/110—100. During work on a bicycle ergometer (the load was for 4 minutes 300 kgm/min. and for 2 minutes 480 kgm/min.) precordial pains radiating to the neck.

Clinical diagnosis: cardiosclerosis.

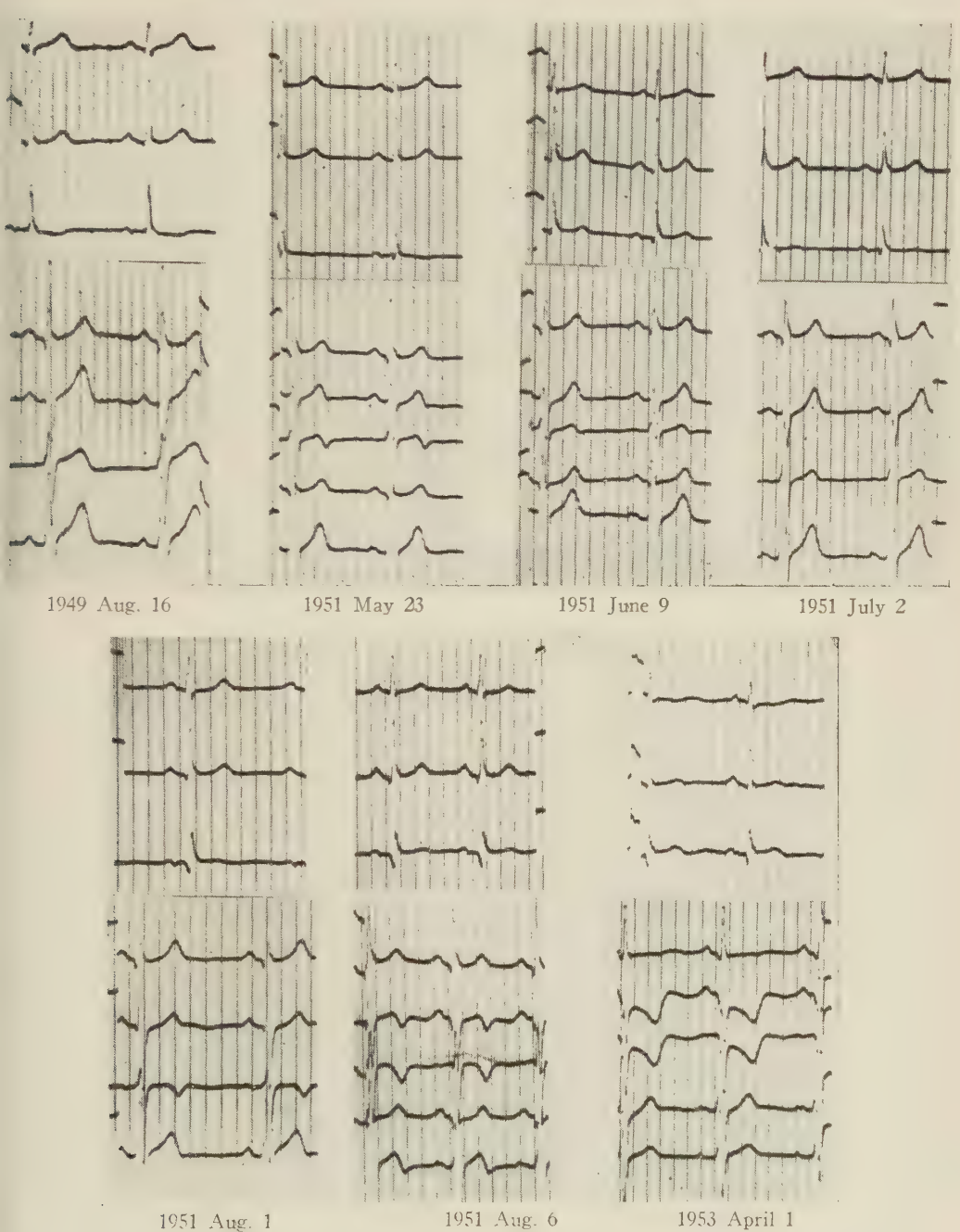


Fig. 1. The leads are in the first, fourth and fifth ECG from above: I, II, III, CR_7, IVR, J and CR_2, in the second, third and sixth ECG: I, II III, CR_7, IVR, J, CR_5, CR_2, in the seventh ECG: I, II, III, CR_7, IVR, J, CR_2, CR_1.

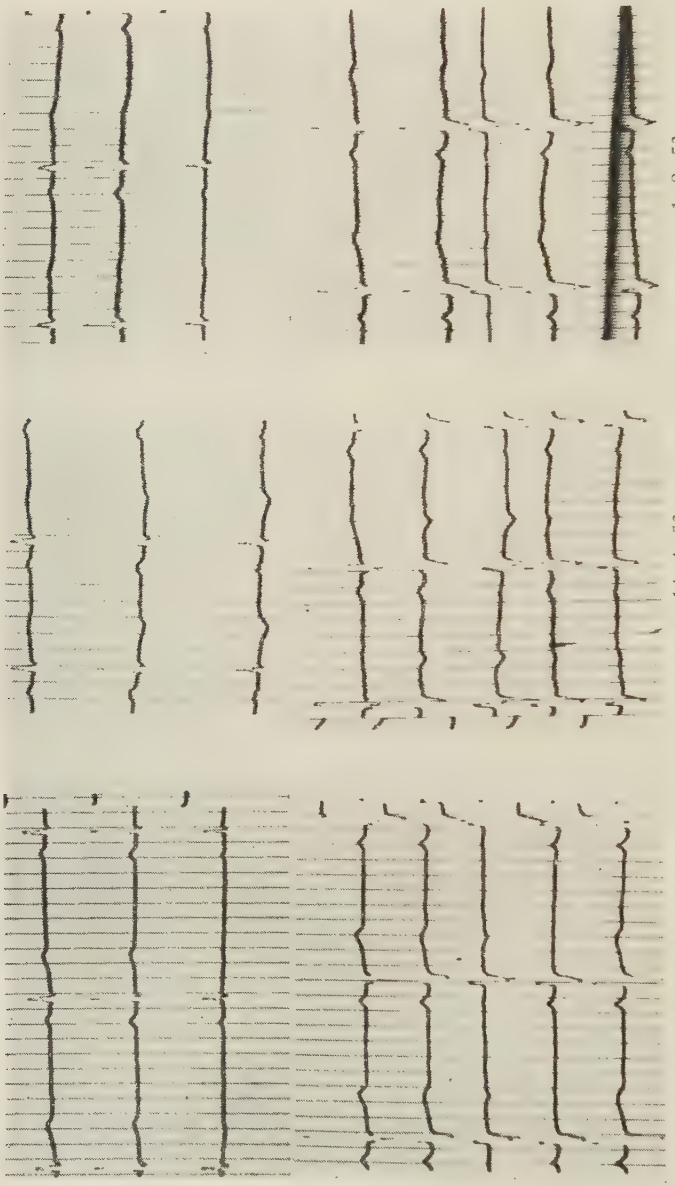
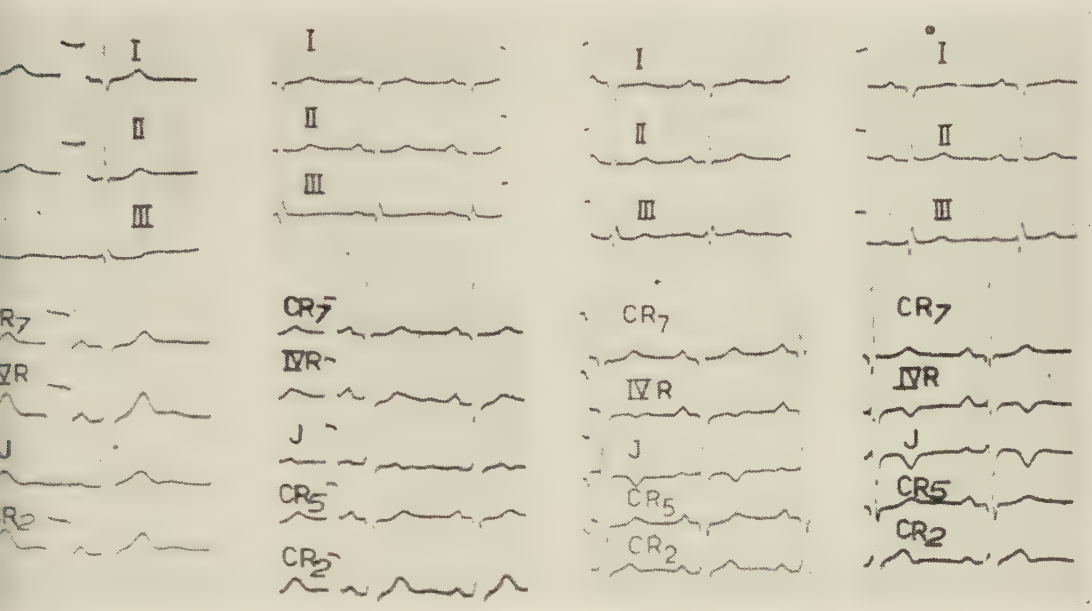
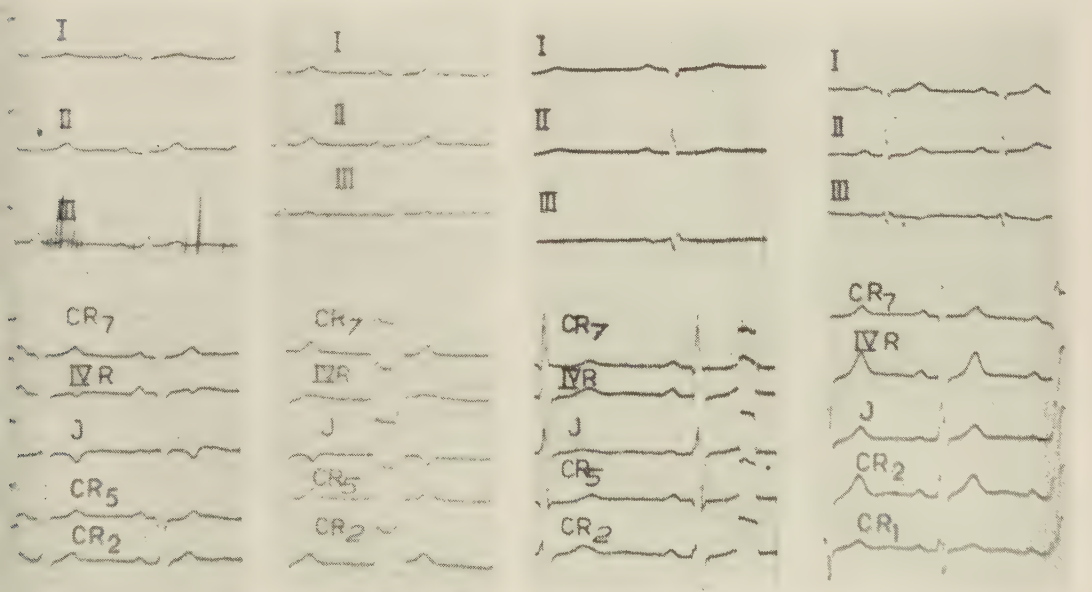


Fig. 2. The leads are in the first ECG from above: I, II, III, CR_7, IVR, J, CR_1, CR_2, in the second and third ECG: I, II, III, CR_7, IVR, J, CR_2, CR_1.



1950 Aug. 9 | 1952 Jan. 18 | 1952 Febr. 11 | 1952 Febr. 15



1952 March 4 | 1952 March 18 | 1952 May 27 | 1953 March 30

Fig. 3. The different leads are marked on the records.

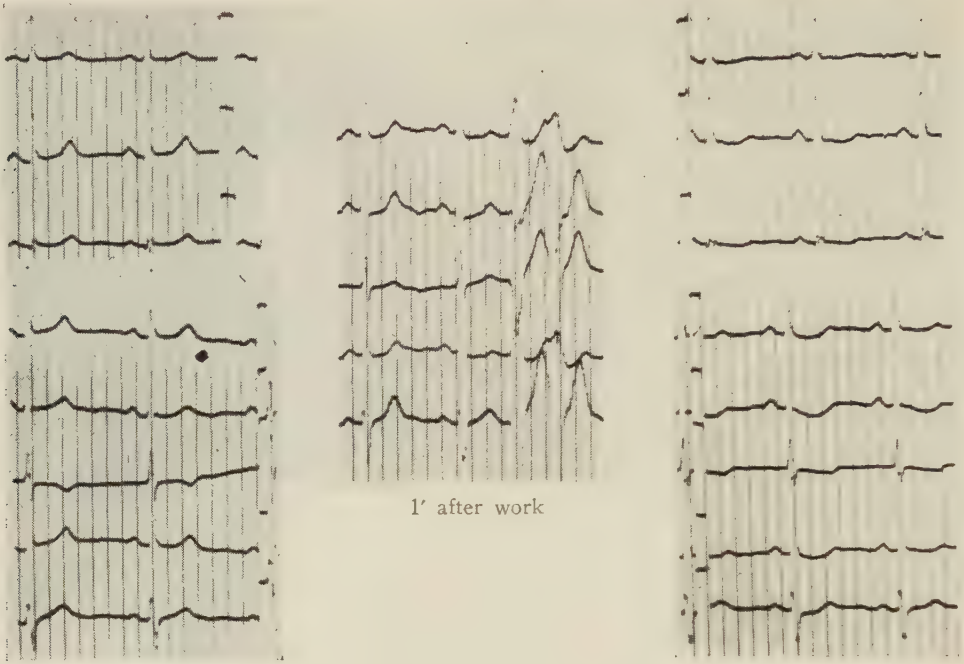


Fig. 4. The leads in the first and third ECG are from above: I, II, III, CR_7, IVR, J, CR_5, CR_2, and in the second ECG: CR_7, IVR, J, CR_5, CR_2.

DISCUSSION

The bipolar chest recording between the apical region and left post axillary line (C_7), called J, evidently gives information of significance in routine electrocardiography.

As the normal T-wave in J is the difference between the larger T-wave in IV R and the lower T-wave in CR_7 it normally has a positive direction. In routine ECG, attention is seldom paid to small variations in the positive amplitude of a T-wave; however, a change of a T-wave from positivity to negativity evokes attention immediately.

Negativity of the T-wave in J can be brought about in two different ways:

(i) an abnormally low T-wave corresponding to the right ventricle in CR_2 reduces the T-wave in IV R, as this lead is a combination of the ECGs of the right and left ventricles.

(ii) a localized myocardial lesion in the apical area can cause a small reduction of the T-wave in IV R, the reduction being not large enough to make the T-wave definitely pathological and without changing the T-waves in other leads.

The first alternative is not within the scope of the present investigation, which deals with a study of the early diagnosis of relative changes in the T-wave in IV R as an isolated phenomenon.

As was shown in a previous investigation a material of 33 patients (Carlsten 1952), T-wave in lead J more negative than ½—1 m is suspect as being pathological. It was so stated that the pattern in question (nor-al ECG with the exception of a more or ss negative T-wave in J) is found in a utine material in about ½—¾ per cent. ne present investigation is an extension of e previous one and the results obtained nd to confirm the conclusions drawn.

A negative T in J as an isolated ECG enomenon can be found in patients with-t symptoms; more frequently, however, fferent subjective and objective signs of art disease are to be found. The results the present paper indicate that negativity the T-wave in J of more than 1 mm = 1 mV is, as a rule, present in clinical heart disease. A less pronounced negativity of the T-wave in J may be a sign of a pathological change, though the majority of cases show normal findings. For these reasons a negative T-wave in J should not be disregarded, for repeated ECG at short intervals may show progressively, definitely pathological patterns of the ECG.

Grewin (1948) observed a higher incidence of negative T-waves in J in women than in men and stressed that this might be due to the breast. In the present material no certain difference between the sexes was found. In other respects the results are in agreement in the main with Grewin's.

The results of the present investigation suggest that the bipolar recording between the apical region and the left posterior axillary line should be used routinely in ECG.

SUMMARY

1. The negative T-wave of the bipolar est recording J, between the apical region d the left posterior axillary line is studied 116 cases showing an otherwise normal CG.

2. Of the material, 40 cases were con-lered normal and the negativity of T in J these cases was found to be 0.38 mm, SD: 8 for female and 0.30 mm, SD: 0.20 for le subjects.

3. 76 cases with probable heart disease showed a negativity of T in J of 0.94 mm, SD: 0.51 for female and 1.26, SD: 0.79 for male subjects.

4. A negativity of T in J of 1 mm = 0.1 mV or more is strongly suspect of being pathological. A less pronounced negativity might be a pathological sign and should always be followed up.

5. Bipolar chest leads should have a place in routine ECG.

REFERENCES

ngtsson, G.: Om värdet av den parasternala avledningen (I. IV. sin.) vid kombinerade bröstavledningar. Nord. med. *36*, 2462, 1947.

lsten, A.: Värdet av bipolär bröstavledning i rutinelektrokardiografi. Nord. med. *50*, 1682, 1953.

Drury, A. N. & Iliescu, C. C.: The electrocardiogram of clinical fibrillation. Heart *8*, 171, 1921.

Grewin, K. E.: Some supplementary leads in clinical electrocardiography. Stockholm, 1948.

Hill, A.: Genesis of normal electrocardiogram. Brit. Heart. J. *8*, 147, 1946.

Lepeschkin, E.: Modern electrocardiography, Baltimore, 1951.

Nehb, W.: Zur Standardisering der Brustwandableitung des Elektrokardiogramms. Klin. Wchnschr. *51,* 1807, 1938.

Nylin, G. & Sällström, T.: Three synchronized leads between fixed points on the heart projection on the chest wall. Acta med. Scandinav. *96,* 1, 1938.

Roche, E. H.: Multiple chest lead electrocardiograms and their clinical value. Brit. Heart. J. *7,* 119, 1945.

Sjöstrand, T.: Changes in the respiratory orga of workmen at an ore smelting works. Ac med. Scandinav. Suppl. *196,* 687, 1947.

— Bestämning av fysiska arbetsförmågan såso klinisk undersökningsmetod. Sv. Läkartidn. *4* 349, 1950.

Trendelenburg, V.: Systematische Methodik d örtlichen Ableitungen. Ztschr. ges. exper. Me *94,* 133, 140, 1934.

Wahlund, H.: Determination of the physical wor ing capacity. Acta med. Scandinav. supp *215,* 1948.

ERYTHROPHAGOCYTOSIS IN CLOTTED BLOOD

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(Received for publication Dec. 12, 1953)

The phenomenon of red cell phagocytosis s attracting great interest at present, especi-lly within the field of immuno-hematology. arge numbers of red cells are probably estroyed by phagocytosis (Wasastjerna 953) in certain forms of immuno-hemolytic nemia. Zinkham & Diamond (1952) and Baumgartner & Müller (1952) have worked ut special methods for revealing red cell hagocytosis *in vitro* as an aid to diagnosis f hemolytic anemia. Recently, Wright, Dodd, Brandt, Elliot & Bass (1953) de-cribed a highly sensitive method for study f phagocytosis of red cells *in vitro*, a method hich is probably too intricate for clinical se. They cultured macrophages from rabbit pleens and examined to what extent human rythrocytes are taken up by these cells, and ound that the red cells of healthy people ere but slightly phagocytized. There was vely phagocytosis, however, when erythro-ytes of sick people were brought to the nacrophages. The "phagocytic index" was reatly raised, not only in acquired hemolytic nemia, but in hereditary spherocytosis as ell, and in a variety of other diseases such s neoplastic conditions and infections of arious kinds, even in common colds.

Early in 1953, a slightly modified form f Magath & Winkle's (1952) method for emonstrating L. E. cells was taken into use t the Maria Hospital. On study of the smears, neutrophils containing ingested red cells were sometimes found. As this was not a regular observation, but occurred in a few smears only, we considered it interesting to find out in what cases erythrophagocytosis does occur. The cases in which L. E. cells are sought constitute a series of selected patients with symptoms common in disseminated lupus erythematosus. Hence smears of blood from patients with different diseases of healthy persons had to be made.

METHOD

About 10 cc venous blood was allowed to clot and stand at room temperature for 90 min. The clot was pressed through a screen, and the liquid obtained was put into a thin tube and centrifugated for 10 min. at a speed of about 2500 r.p.m. Then the tube was allowed to stand for 5 min. in water at a temperature of 37° C. The buffy coat was smeared out on glass slides and stained with May-Grünwald and Giemsa stain. The smears were studied under the microscope through an oil immersion objective, the cells were differentiated until 200 neutrophils had been counted, and the number of cells containing ingested red cells was noted.

RESULTS

Smears from 50 persons were examined and erythrophagocytosis found in ten. Only neutrophilic granulocytes, but no monocytes or lymphocytes, were seen to contain red cells. The leukocytes were highly vacuolized in numerous smears. This was the case especially in the positive smears in which

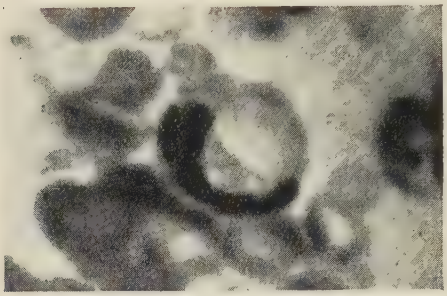

Fig. 1.

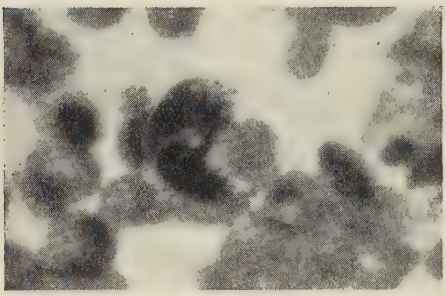

Fig. 2.

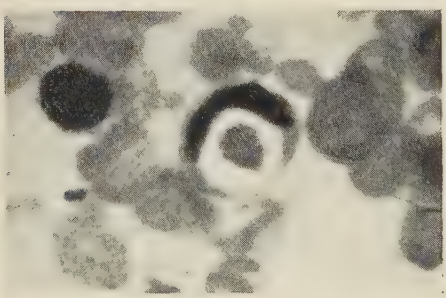

Fig. 3.

Figures 1—3. Neutrophils with ingested red cells.

the granulocytes frequently contained vacuoles the size of an erythrocyte. It is feasible that some of these vacuoles were traces of digested red cells, but, of course these cells were not considered positive. Cells were counted as erythrophages only when they were definitely seen to contain an erythrocyte. On no occasion was more than one erythrocyte observed in each cell. In some of the neutrophils there was a practically intact red cell (Figs. 1—2), while others contained a large vacuole in which a deformed and shrunken red cell was seen (Fig. 3). That there actually was erythrophagocytosis and not a red cell on top of a leukocyte was concluded from the fact that the erythrocyte was always seen to be inside a vacuole. Also the seemingly uninjured erythrocytes were surrounded by a halo, distintcly visible at least on one side.

As seen from the table, the positive cases were patients with severe illnesses, and it is remarkable that high sedimentation rates were noted in all instances except one in which it was moderately increased. The series includes 13 patients with normal sedimentation rates. Erythrophagocytosis occurred in none of these. Nor was it observed in any of the nine cases with small traumatic lesions, in the three cases of peptic ulcer, or in two healthy persons.

COMMENT

Erythrophagocytosis was demonstrated in smears from clotted blood of ten persons with severe illnesses in this modest series of 50 cases. Wright *et al.* found a raised »phagocytic index« in a comparatively large percentage of their vast material, but setting out from the fact that Wright's method is highly sensitive and our method relatively insensitive, there is good agreement in the results obtained. Erythrophagocytosis was pronounced in cases of infectious and neoplastic diseases in both materials.

In the series here reported, erythrophagocytosis was observed in patients with highly increased sedimentation rates. Another phenomenon which runs parallel with the

Table I. *Cases with phagocytosis of red cells.*

Diagnosis	Fever	B.S.R. (mm/1 hr.)	Number of phagocytes per 200 neutrophils
Cancer of the lung	No	94	4
Cancer of the lung	Yes	101	3
Tuberculosis of lymph nodes	Yes	81	3
Bronchial asthma, bronchitis	No	52	3
Cancer of the colon	No	49	2
Pneumonia	Yes	42	1
Chronic bronchitis, fibrosis of the lungs	No	56	1
Myeloma?	Yes	104	1
Cirrhosis of the liver	No	56	1
Spontaneous fracture of tibia, senile osteoporosis	No	24	1

dimentation rate is, according to Hirsch-eck & Woo (1950), the intravascular umping of erythrocytes *in vivo*. It is asonable to assume that there is a common echanism which affects the following three enomena: 1) the rouleaux formation in ood and the sedimentation rate, 2) the travascular aggregation of red cells sludging of the blood") and 3) increased agocytosis of red cells. These three pheno-ena are observed in persons with a variety inflammatory or neoplastic diseases, and may be assumed that they are all affected changes in the surface of the erythrocytes in the surface tension. According to nisely, Bloch, Elliot & Warner (1947), the umped red cells in "sludged blood" are sceptible to phagocytosis. However, in a cent literature review, Wright, Sundhara-ti, Bass & Bunner (1953) state that the ythrocytes taken from persons having rked intravascular sludging do not show increased susceptibility to phagocytosis.

SUMMARY

Clots of venous blood from 50 persons re expressed through a screen, and the ffy coat obtained from the bloody liquid were stained and examined for erythrophago-cytosis. Neutrophilic granulocytes containing red cells were seen in 10 cases, all of them severely ill patients. Most of these patients had a very high sedimentation rate.

REFERENCES

Baumgartner, W. & Müller, U.: Die Hämopsonin-probe in vitro. Helv. med. Acta *19*, 66, 1952.

Hirschboeck, J. S. & Woo, M.: Clinical evaluation of blood "sludge" phenomenon. Am. J. M. Science *219*, 538, 1950.

Knisely, M. H., Bloch, E. H., Eliot, T. S. & Warner, L.: Sludged blood. Science *106*, 431, 1947.

Magath, T. B. & Winkle, V.: Technic for demonstrating "L. E." (Lupus Erythematosus) cells in blood. Am. J. Clin. Pathol. *22*, 586, 1952.

Wasastjerna, C.: Immunohemolytic mechanisms in vivo. Blood *8*, 1042, 1953.

Wright, C. S., Dodd, M. C., Brandt, N. G., Elliott, S. M. & Bass, J. A.: Erythrophagocytosis: standardization of a quantitative tissue culture test and its application to hemolytic, malignant, and infectious diseases. J. Lab. Clin. Med. *41*, 19, 1953.

— Sundharagiati, B., Bass, J. & Bunner, A. E.: Review of the 1952 hematology literature. A. M. A. Arch. Int. Med. *92*, 357, 1953.

Zinkham, W. H. & Diamond, L. K.: In vitro erythrophagocytosis in acquired hemolytic anemia. Blood *7*, 592, 1952.

CLINICAL STUDIES ON THE BUFFER CAPACITY OF THE BLOOD

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(Received for publication Jan. 28, 1954)

The system which maintains the reaction of the blood and body fluids is a manifold combination of different fields of body function. Although the reaction of tissue fluids, different tissues and urine is of great significance in the vital functions, small variations in these reactions do not produce as large and alarming changes in the organism as would be the case if the corresponding changes occurred in the blood.

The reaction of the blood is relatively constant and its variations are reflections either of changes in the composition of the blood itself or of disturbances elsewhere in the body. We know that in incomplete combustion, when *e. g.,* acetone substances remain in the organism, the blood reaction shifts to the acid side, or that, for instance, in tetanic conditions the reaction of the blood is higher than normal, *i. e.,* more alkaline. The same phenomenon is frequently seen in anesthesia, and it can also be produced experimentally by hyperventilation. The respiratory mechanism is, in fact, very closely connected with the blood reaction and the opinion is accepted today that the pH of the blood is an important contributing factor in the mechanism which regulates the function of the respiratory centre (Nims & Marshall 1938; Seevers, Stormont, Hathaway & Waters 1939; Winterstein & Gökhan 1953).

The pH of the blood ranges normally be tween 7.35 and 7.45, the average is usuall reported as 7.41. During anesthesia it ma vary from 7.05 to 7.55, but these extrem values are rarely seen. In some condition of acidosis the blood reaction may be so aci as to give values below 7.

It is a well known fact that blood is strongly buffered solution. A number factors contribute to the maintenance of th buffer system. In the order of importanc they are the following:

1) Hemoglobin,
2) Plasma bicarbonates,
3) Plasma proteins,
4) Plasma and red cell phosphates,
5) Ionic interchange ("chloride shift"
6) Blood lactic acid.

The combined effect of these factors form the quantity which we call the buffer capa ity of the blood. This capacity can also determined by relatively simple methods.

The proportions in which each of the factors influences the buffering properti of the blood were demonstrated by studi carried out by Kilpi in 1952. He was ab to reach the same result by calculations ar

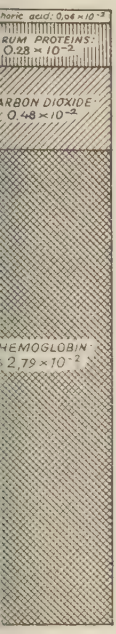


Fig. 1. Diagram showing the factors influencing the buffer capacity of the blood and their mutual relationship at pH 7.3 and hemoglobin concentration of 16 per cent. The dominant share of hemoglobin in the buffer system is seen from the column.

rimetric determinations, especially at a od pH of 7.3. According to Kilpi [1] the ffer capacity is 3.61×10^{-2} when the blood moglobin concentration is 16 per cent. ig. 1).

It is noteworthy that the greatest proporn of the buffer action is produced by hemobin, the factors of next importance being rbon dioxide and proteins. The influence phosphoric acid and other factors is inificant.

The interesting fact is also noted that the arbonate determination used in the usual ical control of the fluid balance accounts only about 13 per cent of the total buffer acity of the blood. However, the bicarate determination is regarded as the test ich indicates the blood acid-base equilibrium and its fluctuations. Even when the effect of serum proteins is added to this, we usually obtain an idea of only about 21 per cent of the total buffer capacity of the blood.

In fluid balance studies, attention has not so far been paid to the total blood buffer capacity. The writers have therefore made determinations of this capacity in connection with thoracic surgical procedures.

Up to the present these determinations have been carried out in 30 operated cases both before operation and several times after operation (Table I and Fig. 2). Two operations were performed in three cases and it was thus possible to observe in these cases the variations in the buffer capacity for over two months (Fig. 3).

The series of medical cases used for comparison consisted of 15 patients with various diseases with which anemia was associated (Table II).[1]

The blood pH was determined potentiometrically, using as comparison a buffer solution of known pH. A potentiometre accurate to 0.01 pH unit and a glass electrode were used.

For accurate determination of the pH, a thermostat (38° C) is necessary and contact of the blood sample with air during drawing must be avoided (Rosenthal 1948, Wilson 1951). As the buffer capacity is calculated from the pH differential, these conditions were not observed, since according to Kilpi (1953) they are not of significance in determining the buffer capacity.

The determinations were made with 5 ml of defibrinated blood diluted with 0.1—N NaCl to make 50 ml. To avoid ketolysis the blood was tested immediately after being drawn or was

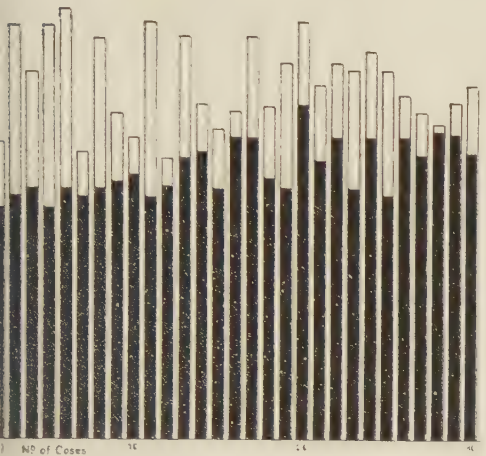
The authors gratefully acknowledge the kind stance of Professor Kilpi in questions relating chemistry.

[1] The authors express their thanks to Professor I. Vartiainen, who placed the comparative material at their disposal at the Second Medical University Clinic.

Table I. *Buffer capacity and hemoglobin values in 10 surgical cases before operation and during postoperative period.*

Ds = postoperative day on which buffer capacity determination was made. B.C. = buffer capacity value on day stated ($\times 10^{-2}$). Hb = hemoglobin value on day stated.

Case 1 Lobectomy	Ds	Before oper.	At oper.	1	6	11	17	23	29	37(1)	43(7)	50(1
	B.C.	3.13	2.63	1.88	1.75	1.72	2.63	2 94	2.86	2.44	2.17	3.4
	Hb	11.35/ 80		8.9 / 63		7.2 / 52	9.55/ 67			9.75/ 68	9.55 67	
Case 2 Commissurotomy	Ds	Before oper.	At oper.	1	8	14						
	B.C.	2,44	2.04	2.86	1.92	2.13						
	Hb	10.0 / 70		11.8 / 77	9.29/ 65	9.19 64						
Case 6 Lobectomy	Ds	Before oper.	At oper.	5	12	19	48	49(1)	56(7)	63(14)	69(20)	
	B.C.	3.57	2.63	2.08	3.03	3.33	3.57	2.77	2.63	2.94	3.12	
	Hb	14.3 / 86	10.9 / 70	9.55 62	9.7 / 64	10.2 / 66	10.2 / 66	10.2 / 66	10.2 66	10.2 66	10.4 67	
Case 8 Lobectomy	Ds	Before oper.		4	11	18	22	28	29(1)	38(10)	45(17)	
	B.C.	3.33		2.27	2.38	2.44	2.70	2.56	2.08	2.04	2.84	
	Hb	11.7 / 72		9 2 / 59	8.9 / 57	7.8 50	7.6 49	7.8 / 50	7.8 50	8.3 / 54	8.0 / 51	
Case 10 Pneumectomy	Ds	Before oper.		1	2	8	15					
	B C.	2.50		2.44	2 94	2.17	2.70					
	Hb	9.8 / 79			10.4 73	8.3 59	8.1 57					
Case 13 Lobectomy	Ds	Before oper.		1	4	10	15					
	B.C.	3.33		2.33	2.38	2.94	3.12					
	Hb	13.1 / 84		10.7 / 69	9.55 62	9.4 61	10.0 / 65					
Case 15 Pneumectomy	Ds	Before oper.		1	8	14	21					
	B.C.	2.56		2.33	2.08	2 38	2.38					
	Hb	10.7 / 69		9.55/ 62	7.2 46	7.4 / 48	8.9 57					
Case 19 Pneumectomy	Ds	Before oper.		1	2	9	16	20	26			
	B.C.	3.12		2 94	2.27	2.08	2.50	2.63	2.86			
	Hb	9.55 62			9.1 58	9 2 / 59	10.0 65	9.55/ 62				
Case 20 Pulmonary resection	Ds	Before oper.		1	7	13	20					
	B.C.	3.45		2.94	2.80	2.77	3.33					
	Hb	12.1 / 79		11.35/ 73	11.0 71	11.0 / 71	11.2 / 72					
Case 23 Lobectomy	Ds	Before oper.		2	7	13	19					
	B.C.	3.03		2.08	2.38	2.38	2.94					
	Hb	11.6 / 75		10.0 / 65	9.2 / 59	9.1 58	9.55 62					



. 2. Diagram showing the buffer capacity values C.) of the entire surgical series before opera-. Shaded portion of columns indicates the lowest buffer capacity level during the postoperative period.

red in the refrigerator, which inhibits ketolysis. e added acid was 0.1—N HCl. The buffer solu-is used as controls were acetate buffer pH 6.5 biphthalate and borax buffers pH 4.004 and 3 respectively. Calibration against a known fer solution was made before and after the ermination.

When the dissociation constants (K_i) and the centrations (C_i) of the acid-base mixture com-ents are known, the buffer capacity (B. C.) can theoretically calculated for the different pH ues (H^+) according to van Slyke's formula, as ows:

$$\frac{d\ (B)}{d\ pH} = B.\,C. = 2.303 \left\{ \sum_i \frac{K_i C_i [H^+]}{(K_i + [H^+])^2} + [H^+] + [OH^-] \right\}$$

$$d\,(B) = \frac{n \times \triangle a}{v}$$

normalcy of added acid or base.
amount of added acid or base.
volume of solution.
$H = pH_1 - pH_2$ pH_1 = measured pH.
= measured pH after addition of acid or base.

The observations described below were made from the determinations in the surgical cases (Table I and Fig. 2: for practical reasons the table includes the determination of 10 cases only).

The buffer capacity of the blood declined postoperatively in 27 cases. In 3 cases a definite rise occurred postoperatively; this rise was clearly related to the large amount of blood transfusions given during operation. In these cases, also, the buffer capacity dropped below is original value 3—5 days after operation.

As a common feature it was observed that where a patient's blood had a relatively high buffer capacity already before operation, the fall postoperatively was smaller, or, if the drop was great, the buffer capacity increased again relatively quickly.

The lowest buffer capacity level was seen 2—10 days after operation. In a notable proportion of cases it declined by as much as 40 per cent or more (6 cases) from the original value. In 9 cases the fall was over 25 per cent, whereas in 15 cases it was less than 25 per cent from the original value. In the cases in which observation of the changes was possible for a longer period, the buffer capacity reverted to its earlier value or near this value within 3—4 weeks.

Since hemoglobin is of primary importance in the maintenance of the buffer capacity of the blood, a sample for hemoglobin determination was taken at the same time as the other blood samples. As was to be expected, a decline in the hemoglobin curve was usually accompanied by a decline in the buffer capacity curve. However, it seemed that although the hemoglobin level remained relatively low during the second and third postoperative

weeks, the buffer capacity began to rise. This is probably ascribable to blood transfusions, and therefore to the foreign red cells received by the patients after operation. The decomposition of these red cells may leave in the blood or restore to it those components of red cells with which the blood buffer capacity is essentially linked.

The relationship of the buffer capacity curve and the hemoglobin curve was interesting and enlightening in those cases in which two surgical operations were performed (Fig. 3). After both operations the buffer capacity dropped abruptly although no decline was seen in the hemoglobin level in any of the cases after the second operation. It is noteworthy that at the time of the second operation the patients were somewhat anemic. It might be presumed that in the anemic condition the red cell wall in some way becomes more permeable for hydrogen ions. This circumstance would also explain why the medical series (Table II) gave clearly and almost without exception relatively higher buffer capacity values even in cases of severe anemia as compared with the values obtained in cases of surgical hemorrhagic anemia. The patients in the medical series had a long history of anemia and thus their organism had had more time to become adjusted to the condition. The dominant significance of hemoglobin and its components in the buffer capacity of the blood is essentially linked with the exchange of ions through the red cell walls.

We know, furthermore, that 90 per cent of the combining of carbon dioxide occurs by means of the hemoglobin and that only 10 per cent of it is compounded with plasma. The hemoglobin is well adapted for this,

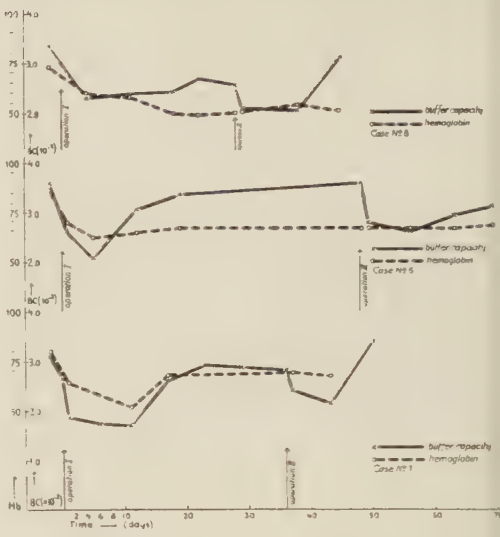


Fig. 3. Diagram showing correlation of the curv for buffer capacity (B. C.) and hemoglobin (Hb in cases in which two operations were performe The second operation in all cases was thoracoplast extrapleuralis. Note the abrupt, almost parall decline in both curves following the first operatio The hemoglobin value remained nearly constant i all cases after the second operation, whereas th curve buffer capacity fell sharply also after the second operation.

firstly because its concentration within th red cells is high and secondly because it isoelectric point, in other words, the reactio at which the maximal loss of its ion chang occurs, lies in exactly the same area as th reaction of the blood. Whenever require by the organism, the blood (hemoglobin possesses this very great capacity to combin carbon dioxide, which at the same tim means also an increase in the buffer actio This takes place without change of the pH when the oxidized blood loses the oxyge and binds carbon dioxide instead.

Table II. *Simultaneous values for buffer capacity and hemoglobin in comparative series of medical cases.*

	Hemoglobin	Buffer capacity
Case 31. Nephritis ac.	4.90/32	1.88×10^{-2}
Case 32. Anaemia aplastica	4.30/30	1.92×10^{-2}
Case 33. Leukaemia	3.80/24	1.61×10^{-2}
Case 34. Leukaemia lymph.	7.06/53	2.44×10^{-2}
Case 35. Syndroma Plummer-Winson	7.02/53	2.63×10^{-2}
Case 36. Spasmophilia	7.3/50	1.88×10^{-2}
Case 37. Anaemia perniciosa	2.9/24	1.43×10^{-2}
Case 38. Hemoglobinuria	3.8/27	1.43×10^{-2}
Case 39. Leukaemia myeloica	5.8/41	2.13×10^{-2}
Case 40. Carcinoma pulm.	4.65/30	1.52×10^{-2}
Case 41. Tumor abdominis	7.2/50	2.50×10^{-2}
Case 42. Anaemia aplastica	6.6/43	2.04×10^{-2}
Case 43. Anaemia hyperchromica	1.95/13	1.43×10^{-2}
Case 44. Febris rheum.	8.5/60	2.63×10^{-2}
Case 45. Leukaemia myeloica	5.15/16	2.00×10^{-2}

Numerous determinations of the alkali serve and plasma proteins were made in nnection with this study but they showed parallelism with the variations in the ffer capacity. This is only natural, as their le in the total buffer system is relatively all, and the changes in their values were rly small even when there were consider- le variations in the blood buffer capacity. or this reason it seems probable that de- minations of the alkali reserve and plasma oteins do not afford a reliable picture of buffer capacity of the blood at a given ne. The hemoglobin concentration is more iable but even this value does not always mply in every respect with the buffer pacity values.

Especially in anemias of long duration, value for the buffer capacity is, according our observations, higher than might be sumed by mathematical calculation based the low hemoglobin values. In this ease, as in many other pathologic con- ions, the organism may have recourse to mpensatory reactions.

The purpose of the buffer capacity of the blood is principally the maintenance of the stability of the pH of the blood. The higher the buffer capacity, the rarer are conditions of acidosis and alkalosis. In this respect the maintenance of the stability of the buffer capacity has chiefly a preventive purpose. We know by experience that post-operative alkalosis or acidosis is frequently very refractory. Efforts should therefore be made to maintain the buffer capacity of the blood near the normal values from the very beginning. This capacity can be raised by means of blood transfusions (Viikari 1953), but sufficient amounts of blood cannot always be given, especially in thoracic surgical cases.

It appears probable that determination of the blood buffer capacity gives a clear picture of the stability of the blood pH level at a given time. The potentiometric determination appears dependable and seems to reflect sensitively even small changes in the buffer capacity. Since this procedure is

relatively simple it is a very suitable test method to be used especially when a reliable picture of the stability of the pH level of the blood is desired.

SUMMARY

The authors review briefly the changes occurring in the blood reaction and the factors maintaining the buffer action of the blood. In the usual laboratory tests which assist in evaluating the fluid balance, the bicarbonate and protein determinations are able to account for only about 21 per cent of the total buffer capacity of the blood.

The authors have carried out determinations of the total buffer capacity in 30 cases involving thoracic surgery. A medical series consisting of 15 patients with various diseases with which anemia is associated was used for comparison.

The blood pH was determined potentiometrically, by comparing the pH of the blood solution with the pH of a known buffer solution. The potentiometre was sensitive to 0.01 pH unit. When the dissociation constants and concentrations of the components of the acid-base mixture are known, the buffer capacity at different pH levels can be calculated theoretically by van Slyke's formula.

In the surgical series the buffer capacity of the blood usually declined postoperatively. It was lowest 2—10 days after operation. In a notable proportion of cases (6 cases) the fall was over 40 per cent of the original value. In 9 cases it was over 25 per cent.

The authors discuss the great significance of the hemoglobin concentration and blood transfusions in maintaining the buffer capacity stable. It seems, however, that during the third postoperative week the buffer capacity values begin to rise even when the hemoglobin values are relatively low. The values for the buffer capacity in the comparative medical series were also relatively higher than would have been presumed on the basis of the surgical series and by mathematical calculations. Numerous determinations of the blood alkali reserve and the plasma proteins showed no parallelism with the changes in the blood buffer capacity values.

The importance of the buffer capacity in the maintenance of the stability of the pH level of the blood is stressed by the authors and they recommend determination of this capacity for the evaluation of the stability of the blood pH.

REFERENCES

Kilpi, S.: Die Dissoziationskonstanten von Hämoglobin und die Pufferkapazität des Blutes. Acta chem. Scandinav. *6*, 475, 1952.

— Zur Pufferwirkung des Blutes. Suomen Kemistilehti B *25*, 36, 1952.

— Personal communication 1953.

Nims, L. F. & Marshall, C.: Blood pH *in vivo* I. Changes due to respiration. J. Biol. & Med. *10*, 445, 1938.

Rosenthal, T. B.: The effect of temperature on the pH of blood and plasma *in vitro*. J. Biol. Chem. *173*, 25, 1948.

Seevers, M. H., Stormont, R. T., Hathaway, H. R. & Waters, R. M.: Respiratory alkalosis during anesthesia. J. A. M. A. *113*, 2136, 1939.

Viikari, S. J.: Changes in the buffer capacity of the blood in thoracic surgical cases. Acta chir. Scandinav. In press.

Wilson, R. H.: pH of whole arterial blood J. Lab. & Clin. Med. *37*, 129, 1951.

Winterstein, A. & Gökham, N.: Ammoniumchlorid-Acidose und Reaktionstheorie der Atmungsregulation. Arch. int. Pharmacodyn. *93*, 212, 1953.

CITRATED OR OXALATED BLOOD IN THE DETERMINATION OF PROTHROMBIN BY THE METHOD OF OWREN

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To prevent the coagulation of the blood determining prothrombin, Owren (1949) d Owren & Aas (1952) used 1/10 volume a 2 per cent potassium oxalate solution. as (1952), however, found that freshly ecipitated calcium oxalate was able to sorb prothrombin as well as proconvertin. According to Aas it cannot be ruled out at even the slight quantity of calcium alate formed by the recalcification in the sma dilutions used in the prothrombin - proconvertin) determinations may under rtain conditions adsorb perceptible quanti- s of the prothrombin + proconvertin con- t from the specimen. In order to avoid y erroneous determinations due to this se, Aas uses sodium citrate to stabilize blood samples.

In order to elucidate the practical signi- ance of this problem in more detail, the hor performed a series of comparative thrombin determinations on blood stabi- ed by oxalate and citrate respectively.

MATERIAL AND METHOD

The studies were carried out on 147 double od samples from 65 patients who could be ected to exhibit major or minor reduction in thrombin activity, either because they were iving dicumarol of suffering from jaundice. The double samples were in each case obtained through the same puncture. The first one was stabilized with 1/10 volume of 2 per cent potassium oxalate monohydrate solution and the second one with 1/10 volume of a 3.1 per cent sodium citrate solution (C_3H_4OH $(COONa)_3$, $2H_2O$).

Double determinations in two or more plasma dilutions were performed. The results, thus, represent the average of at least 4 determinations.

In both procedures, the percentual values were read on a standard curve made on the basis of the author's own blood whose prothrombin content has proved to be constant and very close to the mean value in a large series. For clotting times between 30 and 150 seconds, the two standard curves were rectilinear in the double-logarithmic coordinate system, and their inclination was almost the same.

The stability of the system was under constant control by determination of the prothrombin content in deep-frozen standard plasma.

The fibrinogen preparations used in the determinations were produced from ox blood stabilized with oxalate and citrate respectively.

RESULTS

The results are set out graphically in Fig. 1.

Good conformity is seen between the values obtained by the two procedures. In particular, extremely low percentual values in the oxalated medium were not found to be lower than the corresponding values in citrated medium which had to be expected, if the calcium oxalate, formed by recalcification, adsorbed prothrombin and/or procon-

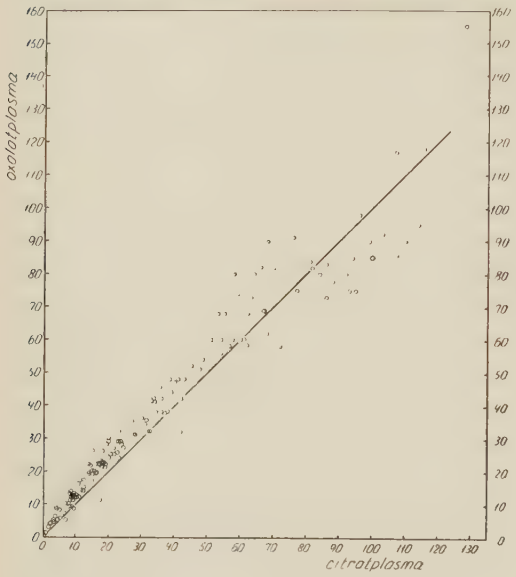

Fig. 1. Relation between prothrombin percentages in oxalated and citrated plasma respectively.

vertin during the determination of the clotting time.

At increasing percentages, particularly those over 50 per cent, the dispersion increases. This dispersion is no doubt due to the standard deviation on the individual prothrombin determination being essentially higher at these subnormal and normal values, where the clotting times are about 40 seconds and where a variation of merely one second will shift the result by 10—15 per cent. At low prothrombin levels, where the clotting times are 100 seconds or more, an alteratio of one second shifts the percentual value b only 1 per cent or less.

The values found in the oxalated mediu are on the average a few per cent highe than the corresponding values in citrate medium. The explanation is presumably tha the inaccuracy of the individual determina tion will prevent an exact fixation of th inclination of the standard curve. An erro in the inclination will entail a correspondin systematic error in the results.

CONCLUSION

The adsorption of prothrombin and pro convertin by freshly precipitated calciu oxalate demonstrated by Aas (1952) doe not appear to affect the result of prothrombi determination by the method of Owre (1949), as the present study revealed goo conformity between the values in bloo stabilized with citrate and oxalate respec tively.

REFERENCES

Aas, Knut: Prokonvertin og konvertin. Diss. Osl 1952.

Owren, P. A.: A quantitative one-stage metho for the assay of prothrombin. Scandinav. J Clin. Lab. Investigation *1*, 81, 1949.

— & Aas, K.: The control of dicumarol therap and the quantitative determination of prothrom bin and proconvertin. Scandinav. J. Clin. Lab Investigation *4*, 201, 1952.

DETERMINATION OF THE FUNCTIONAL RESIDUAL VOLUME BY MEANS OF THE HELIUM DILUTION METHOD

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(Received for publication Febr. 1, 1954)

The use of helium for determination of the tal lung volume was reported by Meneely Kaltreider (1941) and Behnke, Feen & Velham (1942). The method has been modi-ed and extensively used; (Meneely & Kalt-eider 1949, Gilson & Hugh-Jones 1949, cMichael 1939, Bates & Christie 1950). It has been used most commonly in con-ection with the closed circuit system. A view on this is given by Gilson & Hugh-nes (1951). The purpose of this investiga-on is:

a) an analysis of the helium dilution ethod with electric gas analysis,

b) a comparison between the hydrogen lution method according to Birath (1944) d the new helium method.

APPARATUS

Spirometer: Fig. 1. A minor model of the rgatz Knipping spirometer is used. The tubing s been cut down to diminish the dead space of apparatus. The wet carbon dioxide absorber the Knipping apparatus has been replaced by dry carbon dioxide filter. The original multi-e patient connection has been replaced by ordi-ry corrugated tubing. The patient is connected these by a 3-way stop-cock. The capacity the *pump* is set at 50 l/min. and ensures an ost instant flush of the appartus to prevent CO_2 reathing. The *pump* used is the original one ivered with the apparatus. The dead space is 3.700 ml. The total volume of the apparatus including the spirometer bell in the position at the beginning of the investigation is 5.000 ml, a suitable figure according to the calculations of Björklund & Dahlström (1952).

Temperature variations have been studied during a period of rebreathing of ten minutes by insertion of thermometers in the system. It was found that the temperature of the spirometer bell increased by one centigrade. The *resistance* to flow of the various parts of the system was adjusted to keep athmospheric pressure at the mouthpiece. To ensure a constant water level in the spirometer a hole was bored in the spirometer jack.

The *katharometer* measures the thermal conduc-

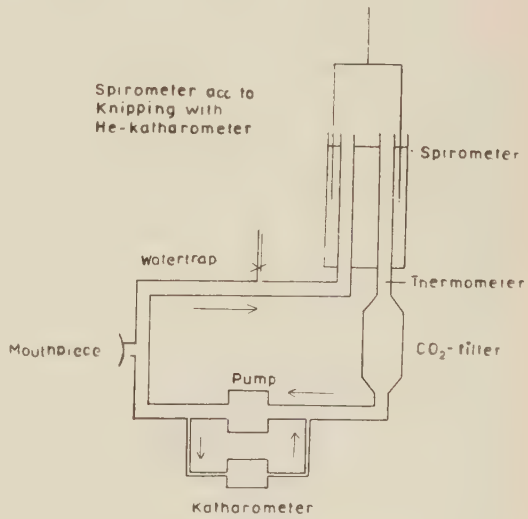

Fig. 1.

tivity of the gas to be analysed. It consists of four glass chambers, two of these sealed with air saturated with water vapour, the other two exposed to the gas to be analysed. The wires are connected in a balanced bridge system and heated by a constant electric current. The heat loss from the wires depends on the thermal conductivity of the gas surrounding the wires. If only one component of the gas, *e. g.* helium, varies the temperature and therefore the resistance of the exposed wires will vary directly with the helium conc. This variance in the resistance causes the balance of the bridge to be disturbed. This imbalance of the bridge is registered on a galvanometer and is proportional to the gas composition. All gases influence the katharometer and in direct proportionality i) to their thermal conductivity and ii) to their concentration. Relative values for thermal conductivity of different gases are given in Table I.

Table I.

Thermal conductivity of various gases in relation to air.

Nitrogen	0.92
Carbon dioxide	0.54
Oxygen	0.99
Hydrogen	6.50
Helium	5.97

Thus the analytical cell is sensitive to variations in a water-vapour, oxygen, nitrogen as well as helium. Since the reference chambers are saturated with water-vapour it is important to maintain a constant water saturation of the gas to be analysed. The O_2 concentration in the circuit must be kept constant. The katharometer is calibrated for 0—20 per cent helium in air and 0—20 per cent helium in oxygen. The value of this special calibration will be discussed later.

The meter is placed in the circuit after the CO_2 absorber and shunted over the pump, in order to avoid a special circulation pump in the shunt. To maintain a constant vapour-pressure in the gas to be analysed, a water-bottle filled with glassbeads has been placed just before the katharometer. The gas reaches the wires only by diffusion which makes them insensitive to the rate of flow through the meter. This makes it possible to have a high rate throught the shunt to get a low timel between the actual alteration in helium concentr tion and the registered one. It is necessary have a constant rate of flow. This has be arranged with a throttle. The time constant this apparatus is about 10 seconds and was adjust with a fixed throttle.

Bates and Christie (1950) have put an extra CO absorber before the meter in order to avoid t error of CO_2 accumulation due to an ineffecti CO_2-elimination. This has been omitted. T reason is discussed under errors of the metho The variation of the temperature in the kathar meter has been studied during rebreathing. T increase after 10 minutes does not exceed half centigrade.

In order to simplify the calculation and avoid t oxygen storage effect (Lassen, Cournand Richards, 1937) the apparatus is equipped with oxygen inlet. Oxygen is administered during t whole investigation to match the oxygen consum tion of the patient.

PROCEDURE

All patients are investigated in the mor ing during standard conditions and in sittir position.

A tracing for determination of the vit capacity and its sub-divisions is taken. The should always be an interval of at least tw minutes between two maximal inspiratio — to ensure midposition again. The mea of three vital capacities is used in the ca culations. The spirometer is flushed with a and a mixture of helium in air is prepare always with the same amount of helium, a and oxygen. When the katharometer h stabilized the current to the bridge checked, the meter deflection and temper ture are registered.

The patient is switched into the circuit the end of an ordinary expiration and at t same time oxygen administration is starte to balance the oxygen consumption. T

xygen flow is adjusted so that the spiro-ram runs horizontally during rebreathing. he helium concentration is read every inute. Before each reading the current to ie bridge is adjusted. This is necessary as ie resistance of the katharometer changes ith the fall in helium concentration. The ebreathing is continued until constant read-gs for three minutes are obtained. When is has occurred it is assumed that there is pproximatly the same concentration of elium in the lungs and in the spirometer rcuit. The patient is then switched off. To void the error of incomplete CO_2 absorb-on, the pump isl eft going for two minutes nd the final reading of the katharometer nd thermometer is taken. The functional esidual volume is calculated from a simple ilution equation. The procedure is technic-ly simple and the total investigation in-uding the calculations does not take more an half an hour.

All volumes are corrected to BTPS, *i. e.* e actual body temperature and surrounding arometric pressure and saturation with ater-vapour.

From the value obtained of the functional sidual volume, *50 cc* BTPS for the dead pace of the mouthpiece are *subtracted.*

Error due to variation in oxygen concen-ation. At the onset of the rebreathing there a mixture of helium in air in the circuit. s soon ast he rebreathing starts, oxygen is n in to balance the patient's oxygen con-mption. It has proved impossible to match e consumption perfectly and as a result e gets small variations of the oxygen con-ntration. To estimate the error introduced this variation a nomogram was prepared owing the influence on the katharometer reading of various oxygen concentrations (20—100 per cent) . Fig. 2. The error was found to diminish with increasing helium concentration. Fig. 3 shows a graph on the correction of the meter for variation in oxygen consumption within certain ranges of functional residual volume. (FRV). From this graph it is seen that the error due to oxygen alteration is small and lies within the error of the katharometer. Correction for oxygen variation within the given limits might consequently be omitted.

Error introduced by helium absorbtion. Meneely & Kaltreider (1941) state that less than 10 ml of helium are absorbed by the body during the period of rebreathing. In a later paper (1949) they doubt the validity of this figure, and believe that the error due to helium absorbtion lies within the error of the katharometer. They continue to subtract 110 ml, an arbitrarily chosen value, from the observed functional residual volume as a correction for absorbed He.

The solubility of helium in blood at 38° C and atmospheric pressure has been determined by Hawkins & Schilling (1936) as approximately 0.0085 cc STPD per ml blood. They have further shown that the solubility of helium in blood follows Henry's law. Thus with an alveolar He concentration of 10 per cent (76 mm Hg), 0.85 cc STPD are dissolved per liter blood. If one calculates with a total blood volume of 5 l, an alveolar He concentration of 10 per cent and no absorbtion to the tissue, 4.25 ml of He are maximally dissolved. If, on the other hand, maximum absorbtion of helium to the tissues occurs, that is the lungs are supplied with blood with a He concentration of 0 per cent, 21.3 ml of He are dissolved in 5 min.

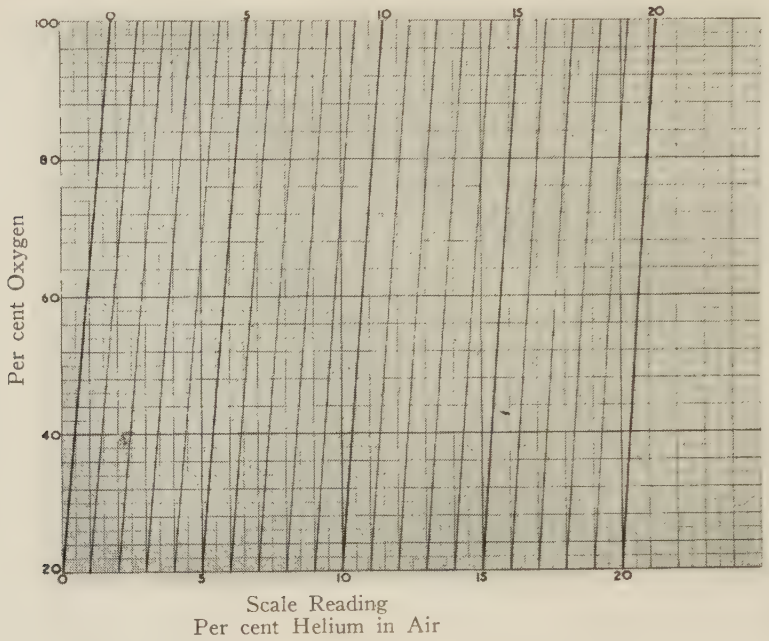

Fig. 2.

This figure corresponds to an increase in the FRV of 200 ml if the total lung-spirometer volume is 7.000 ml. During an ordinary period of rebreathing of 10 min. however, the helium concentration in the alveoli varies between 0 and 10 per cent, that is, thes olubility varies between 0—0.0085 ml He/ml of blood during the same period.

Furthermore a recirculation of blood containing helium occurs. It is impossible to know if the blood is saturated during one passage, as the diffusion constant for helium is unknown. The amount of helium diffused from the blood to the tissues is also difficult to estimate. The deflection of the katharometer for 21 ml of helium, (that is the approximate absorbtion during 5 min. with a constant alveolar concentration of 10 per cent) lies within the error of the katharometer. A correction for helium-absorbtio can only be based on very rough estimations Thus it does not seem appropriate to intro duce a correction factor and correction fo

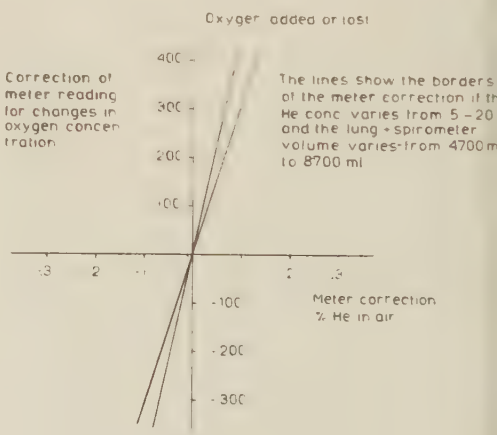
Oxygen added or lost
400
300
200
100
Correction of meter reading for changes in oxygen concentration
The lines show the borders of the meter correction if th He conc varies from 5 – 20 and the lung + spirometer volume varies from 4700 m to 8700 ml
-100
-200
-300
Meter correction % He in air

Fig. 3.

e helium absorbtion has been omitted in e present investigation.

Variation in the current to the bridge in- oduces the largest error involved in this ethod, and it is therefore of great im- ortance to check the current before each eading.

Error due to insufficient carbon dioxide bsorbtion. Carbon dioxide accumulation uring the investigation disturbs the katha- ometer as discussed above and is avoided y replacing the carbon dioxide scrubber etween each subject and by extra carbon ioxide absorbtion after the rebreathing is nished.

Error of the katharometer. The analytical ror of the katharometer has been deter- ined by serial determination on the same elium in air mixture prepared in a Tissot irometer. Three consecutive reading have en made with checking of the current etween each. The last reading in each series three was used for the statistical analysis. fter every three readings the katharometer as flushed with air. Ten such determina- ons were made.

$$M = 15.62 \pm 0.002\ \%\ He$$
$$S.\ D. = 0.09\ \%\ He.$$

Error of katharometer + spirometer. To t an estimation of the error involved when e katharometer is combined with the spiro- eter, the consecutive determinations of the olume of the apparatus were made.

$$M = 3728 \pm 24\ ml\ BTPS$$
$$S.\ D. = 72\ ml\ BTPS$$

Error of the katharometer + spirometer + subject. The total error involved has been determined from 21 duplicate determined from 21 duplicate determinations in 11 normals and 10 cases of emphysema. The standard deviation of a single determination has been calculated according to Dahlberg (1940) and was $\pm$ 89 ml BTPS, which is the figure Birath obtained for the hydrogen dilution method and agrees well with the best results of other investigators.

COMPARISON BETWEEN THE HELIUM AND THE HYDROGEN METHODS

Since the hydrogen dilution method according to Birath was formerly used in this laboratory, it was appropriate to compare the new method with the hydrogen method. For this purpose duplicate determinations with hydrogen and helium were made on 21 cases. The investigations were performed with an interval of half an hour. The mean difference was 0.018 ml $\pm$ 0.098 ml BTPS. S. D. = $\pm$ 0.31 ml BTPS.

Two groups of young men 21 years old without any signs of cardiopulmonary disease were compared. 51 were investigated with the helium method and 102 with the hydrogen method. The following figures were obtained.

Table II.

	Helium	Hydrogen
Number of subjects	51	102
Functional residual volume 1 BTPS	M = 3.35 $\pm$ 0.073 S.D. = $\pm$ 0.52	M = 3.36 $\pm$ 0.055 S.D. = $\pm$ 0.58

From these results it is evident that the lues for functional residual volume obtained with the two methods do not differ significantly.

DISCUSSION

The comparison with the hydrogen method did not show any significant difference between the methods. The helium method has the advantage of beeing more rapid. The introduction of the katharometer in combination with the closed circuit system allows the investigator to folow the helium concentration during the rebreathing. This enables one to continue the rebreathing til the helium concentration is constant. In normal cases this occurs between 5—7 min. With the old method it was necessary to continue the investigation longer than this to be sure that the mixing really was finished.

SUMMARY

1) A description of a modification of th MacMichael method for determination o the functional residual volume is given.

2) The necessary corrections are dis cussed.

3) The errors involved are considere and a comparison between the hydroge method according to Birath and the heliu method according to this modification i made.

4) The accuracy of the method and it time-saving make it suitable for clinical us

REFERENCES

Bates, D. V. & Christie, R. V: Intrapulmonary mixing of helium in health in emphysema. Clin. Science *9*, 17, 1950.

Behnke, A., Feen, B. & Welham, W.: The specific gravity of healthy men. J. A. M. A. *118*, 495, 1942.

Birath, G.: Lung volume and ventilation efficiency. Acta med. Scandinav. 1944. Suppl.

Björklund, O. & Dahlström, H.: On the accuracy of determination of the functional residual air. Scandinav. J. Clin. & Lab. Investigation *4*, 345. 1952.

Briscoe, W., Bechlake & Rose: Intrapulmonary mixing in normal and emphysematous subjects. Clin. Science *10*, 37, 1951.

Briscoe, W.: Further studies on the pulmonary mixing of helium in normal and emphysematous subjects. Clin. Science *11*, 45, 1952.

Dahlberg: G.: Statistical methods for medical and biological students. Allen & Unwin, London 1940.

Gilson, J. C. & Hugh-Jones, P.: The measuremen of the total lung volume and breathing capacity Clin. Science *10*, 37, 1951.

Hawkins, J. & Schilling, C. V.: Helium solubilit in blood at increased pressures. J. Biol. Chem *113*, 649, 1936.

Lassen, H., Cournand, A. & Richards, D. Jr. Studies on the intrapulmonary mixing of gase II. Clin. Science *16*, 1, 1937.

McMichael, J.: A rapid method of determinin lung capacity. Clin. Science *4*, 167, 1939.

Meneely, G. & Kaltreider, N.: Use of helium fo determination of pulmonary capacity. Proc Soc. exper. Biol. Med. *46*, 266, 1941.

— The volume of the lung determined by heliu dilution method. Description of the metho and comparison with other procedures. J. Cli Investigation *28*, 129, 1949.

Standardization of definitions and symbols in resp ratory physiology. Feder. Proc. *9*, 3, 1950.

A NEW ERGOMETER WITH CONSTANT WORK LOAD AT VARYING PEDALLING RATE

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(Received for publication Febr. 1, 1954)

For an evaluation of the symptoms in ardiac and pulmonary diseases it has been ound necessary to perform an analyses dur- ng exercise as a complement to the custom- ry investigations at rest. For this purpose number of ergometers of various kinds ave been introduced. The types most com- nonly used are cycle-ergometers, treadmills nd staircases of various kinds. The ad- antages of the cycleergometer has been ummarized by Sjöstrand (1953). This type f ergometer has been extensively used in his country and several types have been onstructed. A review on this matter is iven by Dahlström (1949).

The ergometers now in use are dependent pon the pedalling rate and if the patient is nable to maintain this, an error is in- oduced which cannot be corrected directly. or the adjustment of the pedalling rate a chometer or a speedmeter is used. Elderly atients and patients with advanced cardio- ulmonary diseases often have difficulties in aintaining a given rate. The error should e avoided if the work load could be kept onstant, independent of the pedalling rate. he present paper is a description of a new pe of cycle-ergometer which fullfils these emands.

APPARATUS

Requirements. As a basis for the development of the ergometer a working range of 200—2000 kgm/min. has been fixed, the effect to be adjustable by steps of 100 kgm/min. Number of revolutions may be varied between about 45 and 70 per minute. The effect must not deviate more than $\pm$ 5 per cent, $\pm$ 10 kgm/min. from the nominally fixed value, if the number of revolutions of the pedalling wheel varies between the limits stated above.

Principle remarks. The present device consists of a pedalling mechanism similar to that of an ordinary cycle. The load consists of a normal separately magnetized D. C. generator. Transmission between pedalling wheel and generator is effected by means of chains. The generator is governed by a regulator and relivers its effect to a loading resistance. Irregularity in the pedalling movement is reduced by means of a flywheel placed on the generator.

General discussion. The aim is to keep the mechanical effect, *i. e.* P = k.n.M constant at varying numbers of revolutions. This is feasible if the regulator takes cognition of number of revolutions and moment, regulating in such a way that the product of

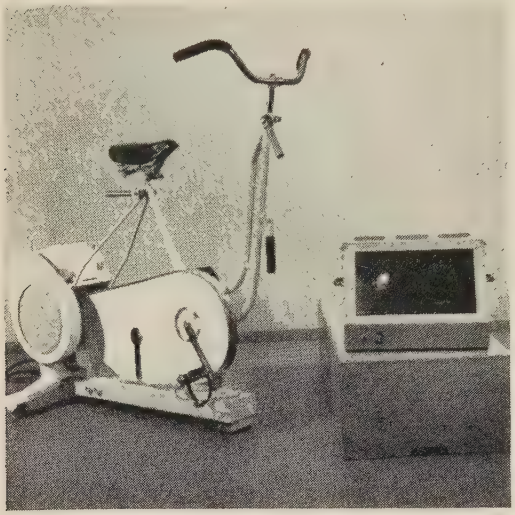

Fig. 1. Cycle ergometer with regulator.

these two magnitudes is kept constant at varying numbers of revolutions. However, great difficulties are encountered in producing a moment-indicator which in a simple way indicates the magnitude of the moment with the desired accuracy, therefore other ways have been chosen for reaching an acceptable solution.

In the present case the problem has been simplified by letting a regulator govern constant output from the D. C. generator, irrespective of number of revolutions within the desired range. The system is diminsioned in such a way that the various loss components are kept within the desired range of variation, with the number of revolutions varying within thes tated limits. By keeping total losses and indicated effect from the generator approximately constant, the effect delivered to the pedalling wheel remains also nearly constant and independent of the number of revolutions. An analysis of the losse involved shows that these can be describe by the following equations.

Friction losses (overall) $P_f = k_1 \cdot n + k_2 \cdot n$

Iron losses $P_{Fe} = k_3 \cdot n^2 \cdot B^2 = \text{constant}$

Load losses $P_b = R.I^2 = \text{constant}$

k_1, k_2, k_3 = constants

n = number of r. p. m.

B = flux density

R = resistance in the armature windin of the generator

I = load current

Since the regulator governs constant out put from the generator irrespective of num ber of revolutions, the electrical losses ar also constant in accordance with the abov equations, for a certain setting of the gene rator. Thus friction losses are the onl sources of deviations in the effect delivere on the pedalling wheel at varying number o revolutions. These losses are reduced b proper dimensioning to a magnitude whic does not involve larger deviations than corre sponding to the desired acurracy within th stated range of revolutions.

The voltage of the generator is kept con stant by feeding the difference in voltag between a reference voltage indicator an the generator voltage to an electronic ampli fier which governs the current to the fiel winding of the generator. In doing so th regulator strives to keep the difference be tween generator voltage and referenc voltage at zero.

Calibration of the ergometer. The calibra tion is performed by driving by a pendulu

ierator with a direct connection of the er to the pedalling wheel of the ergo- ter. Thus all losses are included in the asured effect. The effect delivered to the lalling wheel is taken as a function of reference voltage at 60 r. p. m. of the lalling wheel, and then the reference tage indicator is adjusted to the voltages responding to the effects 200, 300 .... . 2000 kgm/min. The effect is adjusted a switch which selects the voltage corre- nding to the desired effect. A check of deviation at 45 and 70 r. p. m. is then ried out at all loads.

The regulator unit is equipped with an meter measuring the magnetizing current. this ammeter the range is indicated with- which the pointer should be kept. This responds to the permissible range of re- utions. In addition the ergometer is equip- l with a speedometer as a guide for the ient to keep the pedalling rate.

Work ranges. Economic dimensions neces- te a raising of the lower limit of revolu- ns at maximum loads. The work range is refore indicated in the following table:

B	n
kgm/min.	r. p. m.
200—1700	45—70
1800	48—70
1900	51—70
2000	54—70

Accuracy of the ergometer. Dimensioning gnitudes for the ergometer are maximum load at minimum number of revolutions, determining the type size of the generator, and minimum load at maximum number of revolutions, directly determining permissible magnitudes of friction losses. If the range of load and range of variation of number of revolutions is decreased, the limits of errors may also be made more narrow. The accuracy stated in the foregoing of ± 5 per cent, ± 10 kgm/min. is observed at all loads 200—2000 kgm/min. and is thus independent of the ergometer being operated during longer or shorter periods. It may be stated that the effect is constant during the whole pedalling movement, even in cases of very different degrees or irregularity This accuracy satisfies the conditions fixed under "Requirements" but may be increased further if required. However, this necessitates larger sacrifies in material and greater precision of all the details involved, thus involving a substantial increase in costs.

The ergometer is of robust design and its electrical equipment consists of standard parts exclusively, which is advantageous from a service point of view.

SUMMARY

1. The problem of constructing a cycle ergometer suitable for all kinds of patients is discussed.

2. The requirements for such an cycle ergometer are given.

3. An ergometer with constant load at varying pedalling rate which fulfils these requirements is described.

4. The errors involved are discussed.

REFERENCES

Benedict, F. G. & Catcart, E. P.: Muscular work. Carnegie Inst. Wash. Pub. 187, 1913.

Dahlström, H.: En ny cyckelergometer. Sv. läkartidn. 17, 1949.

Krogh, A.: A bicycle ergometer and respiration apparatus for the experimental study of muscular work. Scandinav. Arch. Physiol. *30,* 375, 1913.

Sjöstrand, T.: Changes in the respiratory organs of workmen at an ore melting works. Acta med. Scandinav., Suppl. 196, 687, 1947.

Sjöstrand, T.: Arbetsprövning vid hjärtsjukdo Nord. med. *40,* 1353, 1953.

Tuttle, W. W. & Wendler, A. J.: The constru tion, calibration and use of an alternating cu rent electrodynamic bicycle ergometer. J. La & Clin. Med. *301,* 173, 1945.

Wahlund, H.: Hjärt-lungfunktionsprövning a Militärfall med hjärtbesvär. Nord. med. *2* 219, 1945.

— Determination of the physical working capacit Acta med. Scandinav., Suppl. 215, 1948.

ENDOGENOUS CO FORMATION AND ITS RELATIONSHIP TO IN VIVO HEMOLYSIS IN CONNECTION WITH EXPERIMENTAL INOCULATION OF STREPTOLYSIN AND HEMOLYTIC STREPTOCOCCI

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(Received for publication Febr. 1, 1954)

The effect of hemolytic toxin (streptolysin) *in vivo* has been studied indirectly by determination of the antibody production (Kalbak 1942; Lagercrantz, 1948, 1950). This work aims at measuring the hemolytic effect of streptolysin *in vivo* by a direct method and thereby contributing to the discussion concerning the role which hemolysis plays in toxic and infective states. Several authors have argued whether or not hemolysis plays a role in the occurrence of anemia in infections (Cartwright & Wintrobe 1952).

Sjöstrand (1949, 1952) has shown that CO is continually being formed in the organism and that it can be derived from hemoglobin during its decomposition to bilirubin. By measuring the CO content in the expired air, intravital hemolysis can be followed in normal and pathological states. In the attempts enumerated below, Sjöstrand's method has been used in the study of CO production in the rabbit after injection of streptolysin, and during infection with hemolytic streptococci.

MATERIAL AND METHODS

The technique has been that devised by Sjöstrand (1949, 1952). The rabbits have been healthy and their weight has varied between 2.4 and 3.0 kg. Anaesthesia has been induced with 0.7 ml of "Dial" per kg body weight. A tracheal cannula has been inserted without significant bleeding. Respiration has been through a Müller's valve. Inspired air has been passed through a filter of hopcalit and in this way been freed of Co. The expired air has been collected in rubber bags, every 10 to 15 minutes for the hours following an injection. In the latter part of the experiments samples have been taken every half to one hour. The expired air has been analysed in a particularly sensitive Co-analyser which makes possible the determination of one part of CO in 5 million parts of air. The quantity of CO is given in mls per minute, a calculation of CO in percentage of the expired air has not altered the conclusions.

The streptolysin has been produced according to Kalbak (1942) whose technique for antistreptolysin determination has also been followed.

RESULTS

The first part of the investigation comprised the study of the effect of streptolysin injected intravenously. The experiment included CO determinations:

1. in two healthy rabbits (Table I; Fig. 1).
2. in six healthy rabbits who were injected intravenously with 3 ml streptolysin (dose according to Kalbak, 0.05 to 0.075 ml — see Table 1 for the tabulation of the representative values and Fig. 1 for mean average curves). In Fig. 2 is given the CO production after

Table I. *CO Production in the rabbit after injection of streptolysin or broth compared with controls (CO ml/min × 100.000)*

Time after Tracheal cannula inserted (hours)	Controls (no injection)		Injection of 3 ml Streptolysin after 2 hours 40 min.						Injection of 0.7 ml glucose broth after 1⅓ hour
	1.	2.	1.	2.	3.	4.	5.	6.	
1	40	68	34	43	28	29	36	-	53
									Inj.
2	35	70	51	48	-	54	-	16	94
					Injection				
3	68	52	47	40	-	-	-	17	106
3½	50	43	57	34	72	52	35	25	99
4	34	39	88	78	156	117	66	26	92
5	34	37	163	125	137	114	87	39	91
6	46	39	175	104	135	92	50	45	83
7	41	40	95	105	95	134	50	42	68
8	36	-	78	105	63	89	-	48	56
9	-	-	92	98	90	87	-	57	50
10	-	-	60	107	96	95	-	-	43
10½	-	-	-	112	98	110	-	-	41

(a) injection of 0.7 ml intravenously of an 18 hour old broth culture of hemolytic streptococci (high streptolysin production), (b) injection of 0.7 ml streptolysin intravenously (dose according to Kalbak, 0.05 ml), and (c) injection of 1 ml streptolysin (dose 0.05) neutralised by excess of antistreptolysin (1 ml). The effect of th injection of 0.7 ml of clear broth ca be studied in Table I.

The second part of the investigation com prised the study of CO production durin infection with hemolytic streptococci. Th bacteria were grown in 8 to 10 ml of glucos

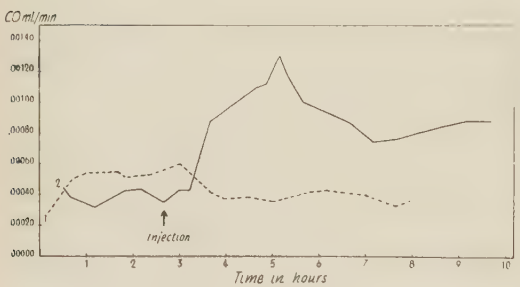

Fig. 1. Formation of carbon monoxide in rabbits after injection of streptolysin (mean values = curve 2) compared with normal controls (mean values = curve 1).

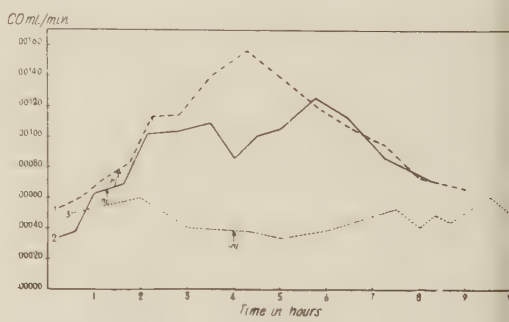

Fig. 2. Formation of carbon monoxide after injec tion of 1) 0.7 ml 18 hour broth of hemolytic strepto cocci. 2) 0.7 ml streptolysin. 3) 1 ml streptolysi neutralized by antistreptolysin in excess.

)th for eighteen hours, they were then shed repeatedly with physiological saline l suspended in about 0.5 ml of physio- ical saline and injected intravenously. The ult was the same with Group A (high eptolysin production *in vitro*) as with oup C, Albany, which is more pathogenic rabbits. In Fig. 3 is given the amount of) production immediately after injection experiments). The temperature of the bits stayed between 38° and 39° C while temperature of the uninfected animals able I, Fig. 1) stayed around 37° C after initial rise. In experiment No. 2 (Fig. 3) rabbit died six and half an hours after ection.

In the experiment, the result of which is own in Fig. 4, the quantity of streptococci s increased to 12 ml of an eighteen hour th culture. The bacteria were washed and ected in 0.5 ml of physiological saline. ie experiment began 20 hours later when rabbit was markedly affected, listless and able to move about. The rabbit died 8 urs after the tracheal cannula had been erted; blood culture showed at that time abundant growth of hemolytic strepto- ci.

In order to study the production of CO a longer time after injection of the bac- ia, the rabbits were infected subcuta- ously. Washed hemolytic streptococci roup A or C Albany) from an 18 hour glucose broth culture (20 ml) were xed with 10 ml of semifluid agar, and ml of the mixture was injected sub- aneously in eight rabbits. Most of the bits were slightly affected by the injec- n, some were listless after 3 to 5 days l all had subcutaneous infiltrations at the

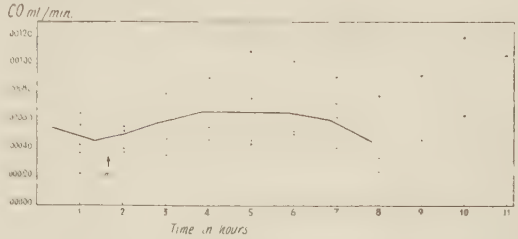

Fig. 3. Formation of carbon monoxide during infection with hemolytic streptococci (injected intravenously). 5 tests. Dots = single values. Curve = mean values.

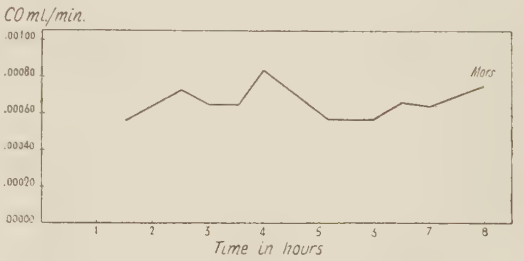

Fig. 4. Formation of carbon monoxide during fulminating sepsis caused by hemolytic streptococci.

site of the injection. One rabbit died after three days (No. 8, Fig. 5). The temperature was usually increased during CO measuring, compared with the control animals. The infected rabbits were injected with the same streptolysin which caused, in the experiment in Figs. 1 and 2, a marked increase in CO production. The amount was in four experiments 3 ml, in one experiment 2 ml and in three 1 ml. The rabbit's antistreptolysin titre was at the end of the experiment between < 25 and 50 and in one case (where infection occurred 7 days earlier) 100 AU/ml.

There was no difference in CO production, before or after streptolysin injection, between the rabbits who had been infected 1 day earlier (Experiments 2 and 8 in

Fig. 5. Formation of carbon monoxide in rabbits infected earlier with hemolytic streptococci and injected with streptolysin. 8 tests. Dots = single values. Curve = mean values.

Fig. 5), and those who had been infected 3 days earlier (Exp. 1, 6 and 7, Fig. 5) or those infected a week earlier (Experiments 4 and 5 in Fig. 5).

DISCUSSION

The method reported by Sjöstrand for the study of the endogenous production of CO in rabbits has, in these experiments, given results which can be reproduced. The values obtained for the endogenous production of CO in rabbits. (Table I and Fig. 1) are in accord with those reported by Sjöstrand (1952). As is evident from Fig. 2, CO production does not increase after injection of biologically neutralized streptolysin. The injection of broth is accompanied by a sharp and relatively short rise in CO production (Table I), probably dependent on the hemolysing effect of the broth.

The injection of active streptolysin (Table I, Figs. 1 and 2) is accompanied by a steep rise in CO production, which was increased during the whole period of the experiment. The difference in reaction patterns between different rabbits is considerable, but in all the experiments values were obtained which were at least three times as high as the values before injection. CO production is increase for a longer time than after the injection o pure hemoglobin (Sjöstrand 1952), fo which reason hemolysis can be expected t continue for several hours after the injectio

In these experiments CO productio (hemolysis) has been increased to only slight degree during infection with hemo lytic streptococci, even if the infection ha been fulminant in type (Figs. 3, 4 and 5) In experiments 1 and 2 (Fig. 3) values wer obtained during several hours which wer about twice as high as those before the in jection. In cases of fulminating sepsis ther was a slight increase in CO production (Ex periment, Fig. 4). Experiments have show that temperature increase in itself is ac companied by an increase in CO productio Whether the observed increase during in fection with hemolytic streptococci is deper dent on the effect of streptolysin or tempe rature rise has not been decided in the pre sent investigation. It seems clear that th intravital hemolysis in these experiment was slight and cannot explain the rabbit' symptoms and death. This agrees with earlie knowledge concerning the effect of strepto lysin *in vitro* (see Kalbak 1942) and wit the opinion that hemolysis does not play an important part in infection with hemolyti streptococci (Cartwright & Wintrobe, 1952)

The difference in the reaction pattern between healthy and infected animals, afte injection with streptolysin, is interestin (Table I and Figs. 1 and 5). The reaso why the infected animals did not react b increased CO production (hemolysis) coul not be due to antibodies, considering tha the difference was already apparent on day after infection, and that the antibod

e was generaly low. It must be related another mechanism.

The method applied here seems to be well apted for further investigations on the ect of streptolysin and its relationship to ection and antibody production.

SUMMARY

The hemolytic reaction to infection with hemolytic streptococci and intoxication with streptolysin has been studied in the rabbit, using Sjöstrand's method for the determination of the intravital CO production.

Injection of streptolysin is accompanied by an increase in CO production, which seems to be a manifestation of increased hemolysis.

During infection with hemolytic streptococci a slight increase in CO production can be demonstrated but, on the other hand, there is an altered reaction pattern on the injection of streptolysin which cannot be explained on the ground of antistreptolysin production.

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REFERENCES

Cartwright, G. E. & Wintrobe, M. M.: The anaemia of infection. A review. Advances Int. Med. *5*, 165, 1952.

Kalbak, K.: Undersøgelser over O-streptolysin og forekomst af O-antistreptolysin i serum. Copenhagen 1942.

Lagercrantz, R.: Hemolytic streptococci and antistreptolysins in healthy blood donors. Nord. med. *40*, 2143, 1948.

— The role of antistreptolysin in scarlet fever and some other infectious diseases. Scandinav. J. Clin. & Lab. Investigation *2*, 152, 1950.

Sjöstrand, T.: Endogenous formation of carbon monoxide in man under normal and pathological conditions. Scandinav. J. Clin. & Lab. Investigation *1*, 201, 1949.

— The formation of carbon monoxide by the decomposition of hemoglobin in vivo. Acta physiol. Scandinav. *26*, 338, 1952.

— The formation of carbon monoxide by the in vitro decomposition of hemoglobin in bile pigments. Acta physiol. Scandinav. *26*, 328, 1952.

A METHOD FOR SPIROMETRIC INVESTIGATION OF THE UPPER LOBE OF THE RIGHT LUNG[1, 2]

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(Received for publication Febr. 9, 1954)

The *principle* of the method is to isolate with a bronchial catheter the air flow of the upper lobe of the right lung from that of the middle and lower lobes, and to make simultaneous spirometric study of each of these two functional units. Because of the normal variations in the anatomy of the bronchial tree the catheter employed is not universally suitable. Its construction, however, complies with the anatomy of the majority of adults. A prerequisite for the use of the catheter, therefore, is that the right main bronchus should be 10 to 15 mm long, and that the distance between the carina and the mouth of the middle lobe bronchus should not be less than 40 mm. The subjects hitherto studied were selected by measuring the relevant bronchial sections at preliminary routine bronchoscopy. The correctness of the catheter's position was checked by bronchography immediately after the spirometric investigation was concluded.

The studies were performed on the "healthy" right lungs of men with pulmonary tuberculosis. Experience to date indicates that the laryngeal passage in the decisive majority of men permits an adequate supply of air to the left lung when the catheter is *in situ*.

The *instrument* is a double-lumen plastic catheter. At body temperature this catheter combines flexibility with good retention of the circularity of its cross-section. The total length of the catheter is about 40 cm, its outer diameter 12 to 13 mm and the thickness of its walls rather less than 1 mm. The lumen is divided into two semicircular channels, each with a cross-section area corresponding to that of a circle 7 mm in diameter. Direct measurements have shown that with the air flow velocities which may be encountered, the fall in pressure in each channel does not exceed 1 cm of water.

By means of a rubber-sheathed, sprung metal hook, which is engaged by the carina as the catheter descends, correct positioning in the bronchial tree is assured. The air channel for both basal lobes (the medial channel) opens into the stem bronchus and is 25 mm longer than the second channel (the lateral). This conducts the upper lobe's air flow and opens into the main bronchus. An inflatable rubber cuff just above the outlet of each channel prevents the passage of air outside of the catheter. The cuffs are inflated through two plastic capillary leads along the outside of the catheter. On their

[1] This investigation has been aided by a grant from the Swedish National Association against Tuberculosis.

[2] Read before the Swedish Association of Phthisiologists, Stockholm, November 28, 1953.

Fig. 1. Diagram of the catheter in the bronchial tree.

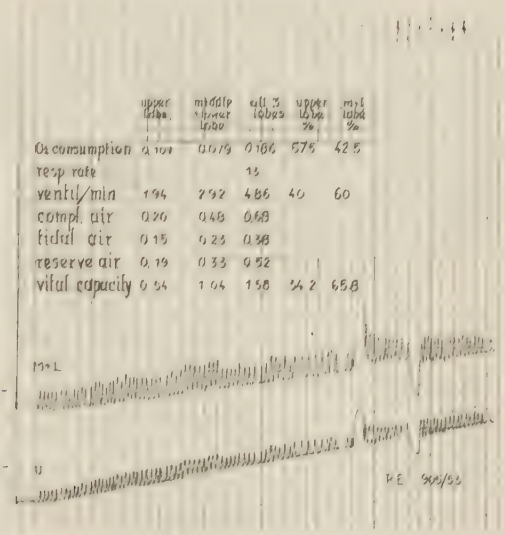
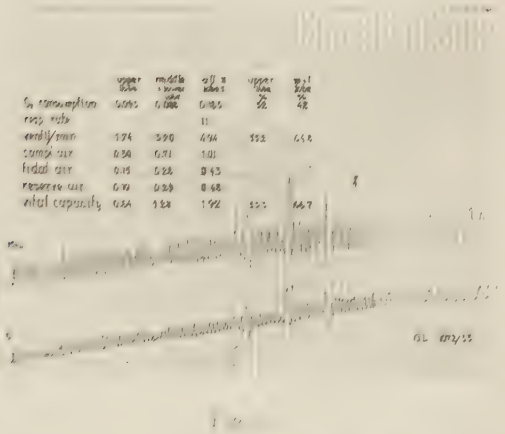


Fig. 2.



Figs. 2 and 3. In each figure the upper curve represents both basal lobes of the right lung. The lower curve derives from the upper lobe.

er parts the leads are provided with con- bags. When the catheter is in position pharyngeal portion is bent forward at an e of 90 degrees or more. To prevent ing the catheter is provided at this point with an outer coil spring. This, however, does not reach down into the larynx and therefore does not limit the rima glottidis.

After the usual anaesthetization of the air passages, the catheter is introduced through

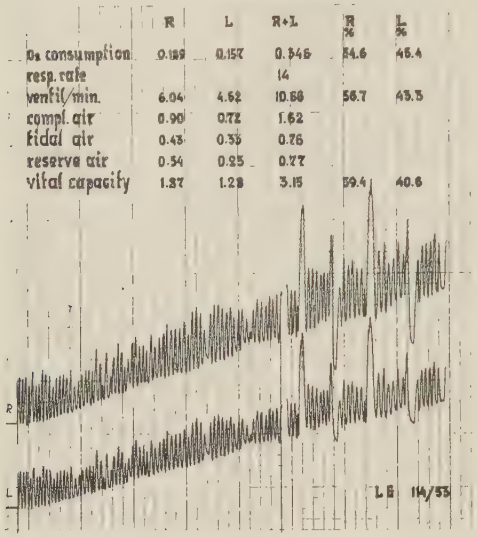


Fig. 4. "Routine" spirometry (according to Björkman (1934), and with Carlens' (1949) catheter of the same patient as in fig. 3, after an interval of a few days. In Fig. 4 the upper curve derives from the right lung. Comparison of the vital capacity of the right lung with the two methods shows 1.92 liters for "lobar", and 1.87 liters for "routine", spirometry.

the larynx under mirror guidance, with the patient seated. A steel stylet gives the catheter the correct angulation. The sprung metal hook is tied to the catheter with a thread. When the lower portion of th catheter has passed through the larynx th stylet is removed and the thread loosened s that the hook is freed. The patient is the placed on the treatment table and the cathet is guided into place under fluoroscop vision. The rubber cuffs are inflated an the catheter connected by two metal adapto at its upper end to a twin spirometer. I this study Björkman's (1934) spirometer constructed on Knipping's principle, wa used. The investigation is then carried ou with oxygen according to Björkman's schem for bronchospirometry.

The spirometer curves in Figs. 2 to 4 ar derived from studies on subjects in the dor sal position.

The aim of the method is to determine th relative normal values for the function o the right upper lung lobe in the erect an recumbent positions, and to make compara tive studies in various diseases of the hea and lungs.

REFERENCES

Björkman, S.: Bronchospirometrie. Acta me Scandinav. 1934, Suppl. 56.

Carlens, E.: A new flexible double-lumen cathete J. Thoracic Surg., *18*, 742, 1949.

TECHNICAL PROBLEMS IN POTASSIUM-SODIUM DETERMINATION WITH THE BECKMAN SPECTROPHOTOMETER

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(Received for publication Febr. 17, 1954)

The purpose of the present work was to amine the following problems in flame-otometric potassium-sodium determina-ns with the Beckman apparatus: —

(1) The influence of differences in cosity and specific gravity between the um dilutions and the known salt solutions the accuracy of the result of measure-nt with this apparatus, which operates hout internal standard.

(2) The influence of varying electrolyte l anelectrolyte concentrations of different ure on the accuracy of the result of asurement.

APARATUS AND METHOD

A Beckman photo-electric quartz spectrophoto-er of the DU-type is used in connection with older flame-aggregate, consisting of a pyrex ss atomizer and an electrically heated atomizing mber (Gilbert, Hawes & Beckman 1950). Pro-e or butane (socalled "Kosan-gas") and oxygen used for the flame, and atomizing is effected h compressed air. In sodium analyses the oxygen ssure is kept constantly at 20 inches of water, potassium analyses at 15 inches of water. The pressure is kept constant at 20 pounds per sq. n, and the cone of the flame measures about m.

n sodium analyses the intensity of the emission neasured at the wave-length 589.3 mμ and with idth of the slit of about 0.04 mm; in potassium analyses the wave-length is 767 mμ and the slit measures about 0.6 mm.

The standard solutions used are 10 solutions with a known content of sodium and potassium chloride in water distilled in glass. The sodium content varies from 0.855 to 1.567 mEq/l, the potassium content from 0.0297 to 0.0547 mEq/l, and the difference between the concentrations of the known solutions thus becomes 0.071 mEq/l for sodium and 0.0025 mEq/l for potassium. The solutions are prepared daily by dilution with glass-distilled water of a stock solution containing 142.3 mEq of sodium per liter and 4.97 mEq of potassium per liter in the form of chloride. The dilution is effected with constriction pipettes (Carlsberg pipettes), and all dilutions are made with the same measuring flask.

Even though the relationship between emission and concentration is practically linear with the procedure employed, it is obvious that when using known solutions with such small intervals in the potassium-sodium content a considerably increased accuracy in measuring is obtained.

In serum or plasma analyses the dilution is 1 : 100, in urine analyses it is adjusted according to circumstances, in potassium analyses it is most frequently 1 : 200 to 1 : 1000, and in sodium analyses it is 1 : 50 to 1 : 200.

RESULTS

Investigation of the influence of differences in viscosity and specific gravity

Beakers with standard solutions as mentioned above and serum dilutions from human subjects, 5 normal persons and 15

patients with different disorders which are known to produce changes in the viscosity of the serum (disorders of the liver and the kidney), were weighed with an accuracy of 0.1 mg. They were then placed under the atomizer for exactly 2 minutes. Afterwards they were weighed again. Pressure, emission during the single experiment and other conditions were constant. The difference in weight before and after atomizing thus expresses the proportion of liquid that has been used up, and the effect of possible differences between the physical state of the solutions can be measured. All experiments were made as duplicate analyses.

Table I.

Serum solutions:		Salt solutions:	
(1) 0.6241	0.6354	(2) 0.6364	0.6397
0.6249	0.6358	0.6386	0.6258
0.6370	0.6375	0.6376	0.6319
0.6424	0.6322	0.6441	0.6338
0.6289	0.6348	0.6553	0.6351
0.6410	0.6382	0.6209	0.6395
0.6313	0.6368	0.6386	0.6428
0.6376	0.6352	0.6346	0.6427
0.6406	0.6410	0.6301	0.6437
0.6391	0.6301	0.6352	0.6204

The table shows the difference in grammes between beakers with serum and those with salt solution before and after atomizing for exactly 120 seconds (every figure is the mean value of 2 determinations).

It appears from Table I that the difference between the average quantities of serum and salt solution used is minimal if there is any difference at all. The standard error in determination of the average difference between the weight of the salt solution and that of the serum solution used cannot be absolutely precisely calculated from the present experiment, as the experiments were not performed in quite a haphazard sequence it is, however, of the order of magnitud below 1 per cent, and there was no measur able difference between the various sera.

According to the measurements per formed, differences in viscosity and specifi gravity between the standard solutions thu exert no influence on the accuracy of th result of measurement under the given con ditions.

Investigations of the influence of variation in the different electrolyte and anelectrolyt concentrations

The Beckman spectrophotometer effe tively shuts out "false" light, *i. e.* the emis sion of light from other wave-lengths doe not influence the photo-cells. But the emis sion of Na- and K-light is not the same fro pure salt solutions and from mixtures, a for instance, the K-emission differs with varying content of Na. This is a well-know phenomenon (see, for instance, Ryssing (1952) extensive review of the literatur and investigations). Investigators of th Beckman apparatus, too, (Mosher, Boyl Bird, Jacobson, Batchelor, Iseri & Mye 1949) have found that the K-emission in creases after admixture of Na, whereas the found that glucose and phosphate reduce th emission of both Na and K.

To find a quantitative measure of th phenomenon, and to examine whether oth substances might influence this emissio the influence of the following substances o the emission of Na and K has been ex amined: Cl^-, SO_4^{--}, NO_3^-, phosphate, ca bonate, bicarbonate, H^+, Ca^{++}, Li^+, NH_4 iron, and urea, glucose, acetone, acetic aci lactic acid and alcohol.

Finally the influence of sodium on the K-emission and, conversely, that of potassium on the Na-emission were measured.

These investigations were performed as follows: —

Aqueous solutions of the substances mentioned were added in highly varying quantities to known aqueous solutions of potassium and sodium, or known solutions were prepared of Na- and K-salt with the acid residue. The content of potassium was 4.97 mEq/l, and of sodium 142.3 mEq/l. After the usual dilution 1 : 100, the emission of these solutions was then measured against

Table II.

Substance	Conc. of substance added (mEq/l-mg%)	Na recovered in %	K recovered in %
Hydrochloric acid	1) 21.5 mEq 2) 43.0 mEq 3) 86.0 mEq 4) 129 mEq	1) 98.3 2) 100.0 3) 100.2 4) 99.4	1) 100.0 2) 100.0 3) 103.2 4) 101.1
Sulphuric acid	50 mEq	100.3	100.0
Nitric acid	50 mEq	100.0	99.1
Phosphoric acid	1) 30 mEq 2) 60 mEq	1) 99.6 2) 100.4	1) 100.0 2) 100.5
Acetic acid	1) 75 mEq 2) 150 mEq	1) 100.0 2) 99 3	1) 100.0 2) 98.9
Lactic acid	2 mg%	99.6	101.4
Bicarbonate (of sodium)	1) 71.2 mEq 2) 142.3 mEq	1) 100.0 2) 100.2	1) 100.0 2) 98.9
Carbonate (of sodium)	1) 71.2 mEq 2) 142.3 mEq	1) 100.8 2) 99.7	1) 99.6 2) 100.0
Lithium (as chloride)	1) 1.0 mEq 2) 10.0 3) 50.0	1) 99.2 2) 100.0 3) 99.6	1) 100.0 2) 100.6 3) 100.7
Ammonium (as NH_4Cl)	1) 28 mEq 2) 70 mEq 3) 140 4) 280 5) 560	1) 98.9 2) 100.3 3) 100.0 4) 98.7 5) 99.0	1) 98.7 2) 97.4 3) 98.6 4) 99.1 5) 100.0
Calcium (as chloride)	1) 1.25 mEq 2) 3.0 3) 6.0 4) 12.0	1) 99.7 2) 100.0 3) 100.0 4) 98.9	1) 100.0 2) 100.0 3) 100.0 4) 100.4
Magnesium (as chloride)	1) 1.0 mEq 2) 2.0 3) 4.0 4) 20.0	1) 100.0 2) 100.0 3) 98.8 4) 100.4	1) 100.0 2) 100.8 3) 100.0 4) 98.6
Iron (as ferroammonium sulphate)	1 mEq Fe	100.0	98.9
Urea	1) 50 mg% 2) 500 mg% 3) 1000 4) 5000	1) 100.0 2) 101.1 3) 100.0 4) 99.4	1) 100.0 2) 99.8 3) 99.8 4) 100.0
Acetone	2 mg%	100.0	100.4
Alcohol	2 mg%	99.6	100.7
Glucose	1) 50 mg% 2) 100 3) 200 4) 1000	1) 99.1 2) 100.1 3) 99.0 4) 100.0	1) 99.8 2) 99.8 3) 100.0 4) 99.7

The table shows the recovery percentages of sodium and potassium in flame-photometry of known aqueous solutions of the substances (partly as chloride where not otherwise stated, partly as salts with the acid residuum concerned) after admixture of known, varying quantities of the electrolytes and anelectrolytes mentioned.

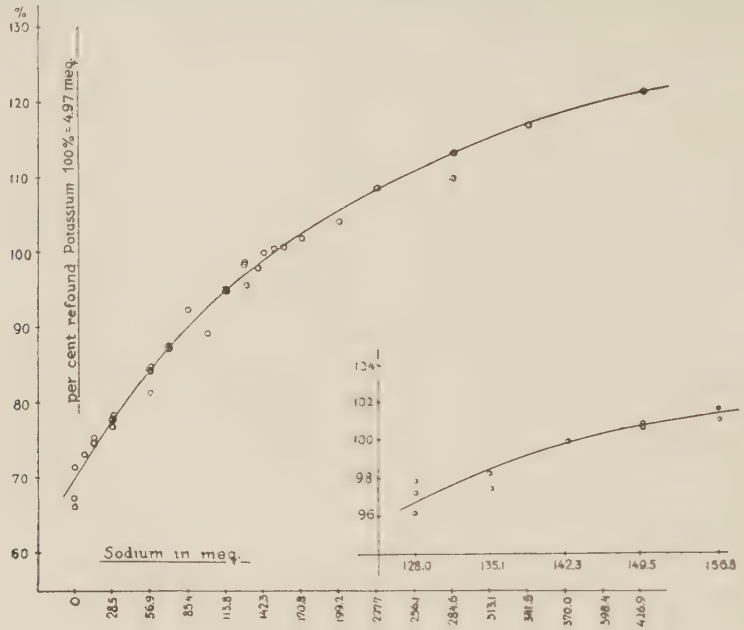

Fig. 1. The figure shows the recovery percentage of potassium with varying sodium content in flame-photometry against known solutions with a constant ratio of potassium-sodium.
The significant range in serum solutions with a sodium content between 128 and 157 mEq/l is shown on an enlarged scale.
All points have been fixed after several duplicate determinations. For the sake of perspicuity, only some points are shown.

the usual, known solutions of sodium and potasium chloride.

All analyses were made as duplicate analyses. The results are given in Table II.

It appears that none of the substances mentioned exerted any considerable influence on either the potassium or the sodium analysis.

On the other hand it appeared that variations in the sodium content influenced the potassium emission to a high degree, since in pure KCl solutions the recovery was only about 67 per cent on analysis against the usual, mixed Na-K standard solutions, whereas the potasium emission increased with increasing content of sodium in the liquid examined. The curve in Fig. 1 shows the recovery percentage of potassium with a rising concentration of sodium.

When the sodium content in the sample is known, the error in the potassium determination can be read (and corrected) directly from the curve. It was found that the influence was determined exclusively by the Na-content, whereas the nature of the acid residue played no part.

Investigations of the dependence of the odium emission on the content of potassium ave the following result: —

If potassium/sodium are present approxiately in the same ratio as in serum, *i. e.* : 50—1 : 30, the sodium emission is not easurably influenced. Only when the ratio ecomes about 1 : 1 does the sodium emission crease by about 5 per cent, but the inuence is rather slight so that with a potasum: sodium ratio of 10 : 1 the sodium mission increases by about 7.5 per cent; ith a ratio of 25 : 1 the emission is 12 per ent, and when the ratio is 50 : 1 it is 15 er cent higher than the emission from the ure sodium solution.

DISCUSSION

As already mentioned, previous investigaors (*e. g.* Ryssing 1950, Mosher *et al.* 1949, Vest, Folse & Montgomery 1952) have also ound that the potassium emission increases hen sodium is added to the solution, but is phenomenon has not been quantitatively etermined with Beckman's apparatus; it is t least not caused by emission of light by odium on the wave-length of potassium. his is proved by the fact that even enorous sodium concentrations do not measurbly influence the photo-cells at the wavength of potassium (767 mμ).

However, in the present experiments it as not posible to reproduce the findings y Mosher *et al.* (1950) that the emission of oth potassium and sodium is inhibited by lucose (Mosher, however, used 2 per cent f glucose) and phosphate; and according to e present results there is apparently no eason to consider urea, magnesium and other substances which Mosher, undoubtedly rather on grounds of principle, adds to his stock solutions. It is possible that Mosher's results are due to increased viscosity and specific gravity of the liquids analysed, as he used serum concentrations which were twice as high as in the experiments reported above. Mosher also added 2 per cent of alcohol to both samples and known solutions, and gelatin to the known solutions to adjust their viscosities to one another. According to the present experiments, this should not be necessary.

To eliminate a possible error by using different standard solutions like Mosher *et al.* (1950) and other investigators is, at best, a troublesome procedure to use, especially in the case of urine analyses where the proportions of the substances vary enormously, even though only potasisum and sodium need to be considered according to the investigations performed.

By establishing a curve for the influence of sodium on the potassium emission the obvious advantage is obtained that one set of standard solutions will suffice for ordinary clinical purposes. The sodium content in the sample should just be determined — or known. Furthermore, it appears from the curve that in routine examinations of serum, in which the sodium content seldom deviates more than ± 15 per cent from about 142 mEq, the fundamental error in the uncorrected potassium determination is rather insignificant. It should be corrected for, but clinically an error in a potassium determination of about 5 per cent at most will hardly play any role. In urine analyses, on the other hand, especially in controlling a salt-free diet, a correction should always be made.

This, of course, should also be the case in scientific analyses, and it will most frequently be safest to use the various standard solutions with approximately the same ratio of potassium: sodium as in the sample.

CONCLUSION

According to the results of the present experiments, one of the main objections to flame-photometry without internal standard, namely that there is a difference between the amount of serum dilution and that of the known solution applied in the flame, apparently plays no part in the Beckman apparatus with the technique used.

Various substances which may be present in biological fluids do not influence either the potassium or the sodium emission, but variations in the ratio potassium: sodium may give rise to an error in the potassium determination. However, correction can be made for this error from a standard curve if the content of sodium is known, and it will hardly exceed a few per cent in serum-plasma analyses.

The sodium analysis, too, shows a similar error; in serum analyses it is minimal (not measurable), but in analyses of fluids (*e. g.* urine) in which the ratio of potassium: sodium is higher, a correction should be made.

REFERENCES

Gilbert, P. T. Jr., Hawes, R. C. & Beckman, A. O.: Beckman flame spectrophotometer. Analyt. Chem. *22*, 772, 1950.

Mosher, R. E., Boyle, A. J., Bird, E. J., Batchelor, T. M., Iseri, L. T. & Myers, G. B.: The use of flame-photometry for the quantitative determination of sodium and potassium in plasma and urine. Am. J. Clin. Pathol. *19*, 461, 1949.

Ryssing, E.: Studier over den flammefotometriske metodes fejlkilder og anvendelighed til bestemmelse af kalium og natrium i blod og urin. (Thesis) Copenhagen 1952 (Dansk videnskabs forlag).

West, Ph. W., Folse, P. & Montgomery, D.: Application of flame-spectrophotometry to water analysis. Analyt. Chem. *22*, 667, 1950.

A BED-SIDE "PROTHROMBIN" METHOD FOR USE IN ANTICOAGULANT THERAPY

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When preparations of the dicumarol type e used in anticoagulant therapy, careful bservation of the prothrombin level is cessary. The reliable methods of determina-on are rather timeconsuming, however, and quire equipment. Hence their use in small spitals without adequate laboratory facili-es is limited. So-called bed-side methods, nich are simple and quick, are therefore eded, but simplification at the expense of liability is not permissible.

Several such methods have previously en described. The majority of those blished (Kato & Poncher, 1940; Schwager Jaques, 1949; Ziffren, Owen, Hoffmann Smith, 1939 and 1940) are based on orawitz's theory that the three factors othrombin, thromboplastin and calcium, nich have been known for a long time, are quired for the clotting of blood. However, is not the plasma prothrombin that is de-rmined, but the sum of all the factors esent in the so-called prothrombin complex rothrombin, proaccelerin and proconver-), the properties of which have been cidated during the past few years (Aas, 52; Owren & Aas, 1951).

Recently a rapid method has been ela-rated by Marbet & Winterstein (1953 a d b) at F. Hoffmann-La Roche & Co. AG.s Laboratory, Basle. Blood is used in this test, and Koller (Marbet & Winther-stein, 1953 a) has stated that this method is fully as accurate as those using plasma. The present writers have studied the method in question and compared it with Quick's well-known prothrombin method and Owren's one-stage method (Owren, 1949 and Owren & Aas, 1951).

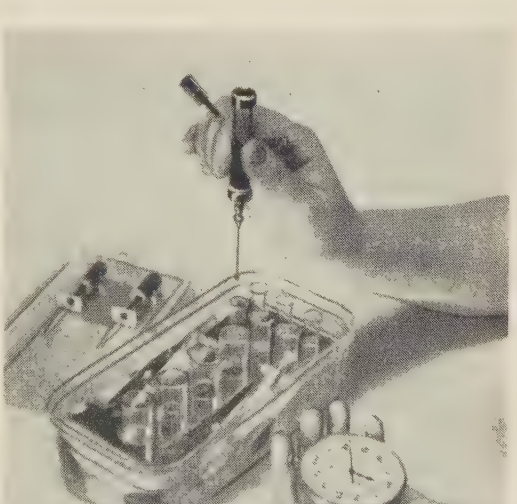

Fig. 1.

The principle is as follows: A certain amount of citrated blood, is mixed with thromboplastin and calcium chloride and allowed to drop from a syringe through an injection needle. The moment of clotting is easily observed, as a drop of blood which has run out remains attached to the point of the needle.

Equipment. Marbet & Winterstein have prepared a set consisting of a plastic box, which serves as a thermostat when filled with warm water, a 2 ml syringe fitted with an injection needle No. 1, a thermometer, and four large and six small test tubes.

Reagents. Sodium citrate solution 3.6 per cent, calcium chloride solution 0.548 per cent $CaCl_2 + 6\,H_2O$, thromboplastin solution "Roche".

Procedure (Fig. 1). The plastic box is filled with water at 25—37° C. 1.8 ml of venous blood is drawn into a syringe with 0.2 ml of citrate solution, and the mixture is squirted into a test tube. 0.7 ml of this citrated blood is drawn into the syringe and this amount is squirted into a test tube containing 0.5 ml calcium chloride and 0.5 ml thromboplastin solution. As the blood is squirted, a stopwatch is started. Immediately afterwards the mixture is again drawn into the syringe, and the piston of this is removed. By pressing with a finger against the opening of the syringe a slight excess pressure is produced, which makes the blood mixture drop out through the needle. When a coagulated drop of blood remains suspended at the point of the needle the watch is stopped and the "prothrombin value" is read from a standard curve or table. According to Marbet & Winterstein, an overdosage of dicumarol (corresponding to a "prothrombin value" under 10 per cent) has been given if the syringe runs out at a temperature of 30—37° C. This test can also be performed with reagents of other brands than the above-mentioned, provided a special curve or table is made.

RESULTS

The series includes 6 cases of cardiac infarction, 2 cases of deep thrombosis in the lower limbs, one case of pulmonary infarction, one of cerebral thrombosis, all of which were treated with anticoagulants. In 6 of these 10 cases concurrent determinations were made during the treatment by Marbet-Winterstein's (M-W), Quick's and Owren's methods; in 4 cases the values obtained by the M-W method were checked only by Owren's method. Furthermore, random tests were performed by the M-W and Owren's methods in 20 cases of thromboembolism

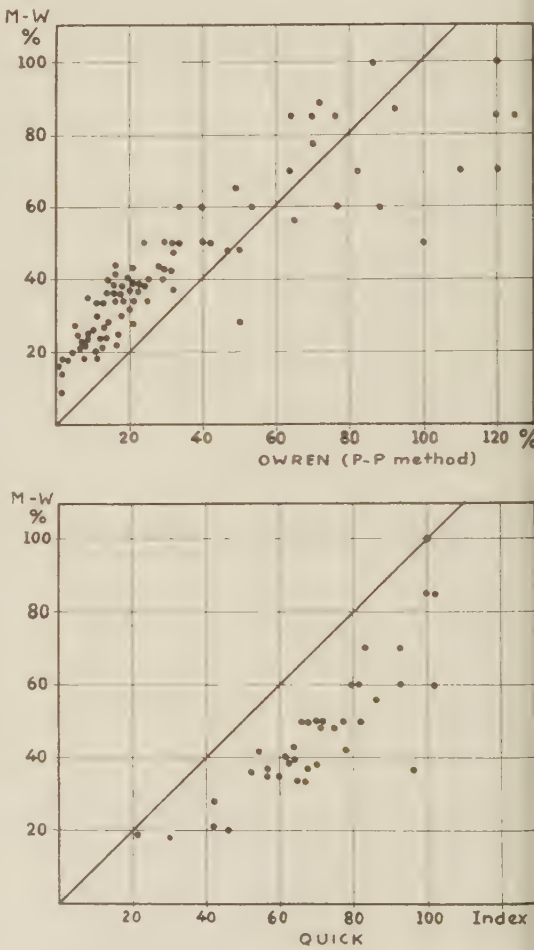

Fig. 2.

ıe total number of determinations by the -W method was 89; all of them were ecked by Owren's method and 38 by uick's method also (Fig. 2).

In Fig. 3 the results obtained in 10 cases Marbet-Winterstein's and Owren's ethods, respectively, are compared. It is und that the agreement between the two ethods is fairly good, and that the curves e essentially similar, even though the M-W lues are, as a rule, somewhat higher. In g. 4 the M-W method is compared with uick's. The parallelism between these lues is less marked, and the two curves

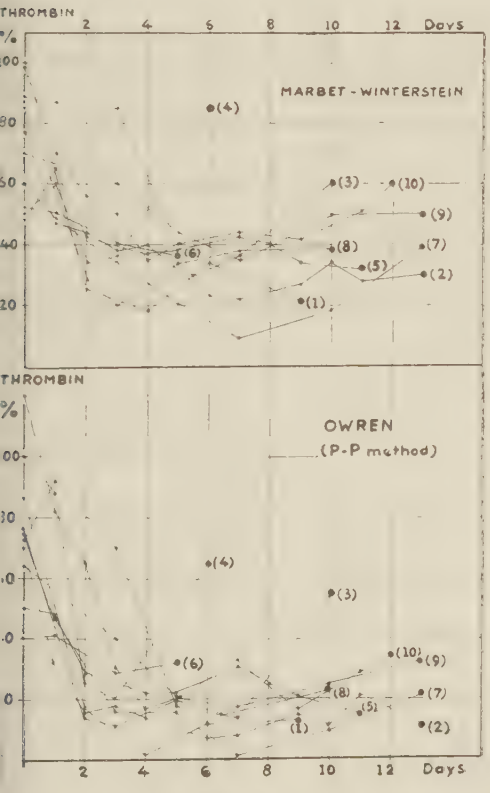

Fig. 3.

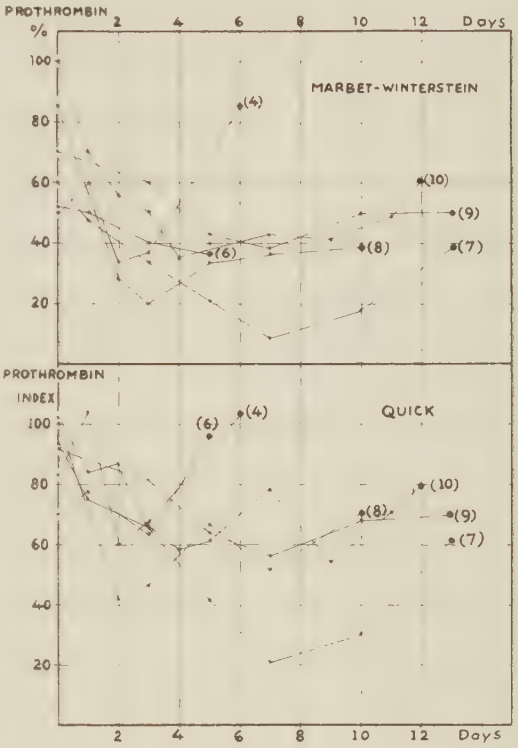

Fig. 4.

run somewhat different courses. The average difference between the two sets of values is about 15 per cent in the former case; in the latter it is about 23 per cent. Fig. 5 illustrates the course in a case of thrombosis under treatment; the correlation between the results obtained by the different methods is striking.

DISCUSSION

The M-W and Quick methods both involve determination of the entire prothrombin complex. The sensitivity of such methods cannot be very high. Nevertheless it appears that the M-W method can be used in practice in anticoagulant therapy. It should, how-

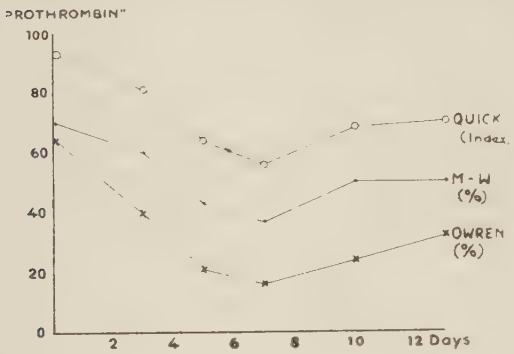

Fig. 5.

ever, be borne in mind that a decrease in one or more factors of the prothrombin complex results in a decrease of the value, as is seen in congenital hypoaccelerinemia or hypoproconvertinemia, and in liver damage. The defects of the method appear in Fig. 2. Increased concentrations of one factor cannot be recorded, any more than by Quick's method. Marbet & Winterstein state that there is overdosage of dicumarol if the syringe runs out at a temperature of 30—37° C, *i. e.* the "*prothrombin* value" is then less than 10 per cent. Such a value (9 per cent) was noted at only one of the 89 examinations performed. On the other hand determination by Owren's P-P method gave very low prothrombin values in as many as 15 cases, *viz.* values under 10 per cent, which has been found to be the risk zone (Fig. 2).

Even though there was no marked correlation in the risk zone between the values obtained by Owren's and the M-W methods respectively, the agreement was otherwise appreciable. The M-W method seems, therefore, to be useful in anticoagulant therapy in cases where it is not possible to determine the prothrombin by more reliable methods owing to their intricacy, and in situations when a rapid decision is needed at the bed-side as to whether dicumarol therapy should be continued or not.

SUMMARY

Marbet-Winterstein's (M-W) bed-side method for use in anticoagulant therapy has been studied in 10 cases of thromboembolism over a considerable period of time and in 20 random cases. Eightynine determinations were made. For the sake of comparison the same number of determinations was simultaneously performed by Owren's one-stage method and 38 by Quick's method. Both the curves of the results and single determinations displayed, as a rule, an appreciable degree of correspondence, in particular when the M-W and Owren's methods were compared. Exceptionally high "prothrombin values" cannot, however, be recorded by the former. It appears, too, as if highly pathological values within the risk zone could not be exactly recorded. The M-W method is useful at the bed-side when it is important to form an opinion quickly as to whether dicumarol therapy should be continued, and in particular in anticoagulant therapy when it is not possible to determine the prothrombin by more elaborate methods. The average difference between this method and Owren's is about 15 per cent. During treatment the "prothrombin" level should thus be maintained at 25—45 per cent.

ACKNOWLEDGEMENT

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REFERENCES

as, K.: Prokonvertin og Konvertin, Oslo 1952.

ato, K. & Poncher, H. G.: The prothrombin in the blood of newborn mature and immature infants. J. A. M. A. *114,* 749, 1940.

oller, F.: Quoted by Marbet & Winterstein. Praxis *42,* 61, 1953 a.

Iarbet, R. & Winterstein, A.: Neue Methodik zur Kontrolle der Anticoagulantientherapie. Praxis *42,* 61, 1953 a.

— — Zur Methodik der Prothrombinbestimmung. Helv. Physiol. Acta *11,* 81, 1953 b.

wren, P. A.: A quantitative one-stage method for the assay of prothrombin. Scandinav. J. Clin. & Lab. Investigation *1,* 81, 1949.

— & Aas, K.: The control of Dicumarol therapy and the quantitative determination of prothrombin and proconvertin. Scandinav. J. Clin. & Lab. Investigation *3,* 201, 1951.

Schwager, P. G. & Jaques, L. B.: A simplified technique for the determination of prothrombin times. Canad. M. A. J. *60,* 258, 1949.

Ziffren, S. E., Owen., C. A., Hoffman, G. R. & Smith, H. P.: Control of Vitamin K therapy. Compensatory mechanisms at low prothrombin levels. Proc. Soc. Exper. Biol. & Med. *40,* 595, 1939.

Ziffren, S. E., Owen, C. A., Hoffmann, G. R. & Smith, H. P.: A simple bedside test for control of Vitamin K therapy. Am. J. Clin. Pathol. *10,* 13, 1940.

COMMENTS ON METHODS IN BIOLOGICAL WORK WITH S^{35}-LABELED COMPOUNDS

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Although the methods for assay of radioactive carbon C^{14} have been discussed extensively in the literature of recent years, similar reports on radioactive sulphur have been infrequent (Young & McCarter 1948, Schweitzer & Stein 1950). The following are comments on methods which we have found useful in biological work with S^{35}-labeled compounds. The reader is referred to the literature for the detailed description of the procedures and discussion of the principles involved.

When working with tracer amounts of radioactive compounds, side reactions, insignificant in work with larger amounts, may produce erroneous results. These side reactions are particularly prevalent in work with sulphur because of the reactivity of this element. It is therefore necessary to check every step in a procedure in order to assure its reliability. For example, in studying the intermediary conversion of cystamine (the disulphide of mercaptoethylamine) to taurine *in vivo* (Eldjarn 1952), the taurine had to be isolated from the bile after acid hydrolysis of the taurocholic acids. Check runs showed that this treatment of 0.1 mg of labeled cystamine in the presence of unlabeled taurine gave on the average a 30 per cent conversion of the cystamine to taurine. After the addition of 200 mg of inactive cystamine as carrier, this conversion was reduced to 3 per cent. The remedy in this example actually consisted of converting the micromethod to a semi-macromethod by the addition of carrier. This technique may also be used to reduce the errors which are frequently encountered when working with tracer amounts.

Sulphur S^{35} is a β-ray emitter; its maximum energy is 0.167 Mev and its half life is 87.1 days. The measurement of soft β-radiation involves many possible sources of error; therefore standardised and easily reproducible counting conditions are necessary.

The chemical form in which radioactive sulphur should be counted to yield the most satisfactory results is a moot question. Several possibilities exist: plating the sulphur-containing compound directly, or plating after oxidation as benzidine sulphate or barium sulphate. In certain cases direct plating may give satisfactory results. Where greater accuracy and reproducibility are desired, one of the other two methods is recommended. One disadvantage with the benzidine sulphate is its sensitivity to the precipitation conditions. Furthermore, the benzidine sulphate method can not be used in conjunction with the sodium peroxide fusion. We have therefore adopted the mounting as barium sulphate, which can easily be precipitated quantitatively and also has the advantage that it can be used with any of the oxidation methods employed in work with sulphur compounds.

THE DETERMINATION OF RADIOACTIVITY IN A SAMPLE

Purification. The compound in question is converted to a crystalline derivative whenever possible. For purification the crystal-

sation is repeated 3 to 5 times after a correct melting point or some other known physical characteristic is attained; preferably two or more different solvents are used successively. Sometimes "recrystallisation to constant specific activity" may be worthwhile but as a routine procedure the above method has proved satisfactory.

For isotope work all glassware should be marked in some way to ensure that equipment used for low activity samples will not become contaminated through contact with samples of high activity.

Oxidation. If one uses barium sulphate ating, the sulphur in the samples must first oxidised to sulphate. Usually an aliquot ntaining sulphur corresponding to approximately 100 mg of barium sulphate is easured out. In cases where the available nount of a sample is limited or its sulphur ntent is very low, one will have to add rrier. Many workers add sodium sulphate rrier after the oxidation. In our opinion ere are advantages in adding an organic lphur compound as carrier prior to the idation, preferably a known amount of unlabeled sample of the substance to be idised. This will minimise some errors, ch as losses due to adsorption to the oxiion equipment. The amount of carrier is ually calculated to give a total of about 0 mg barium sulphate.

For the oxidation the following three produres have been found useful:

1) Sodium peroxide fusion. This method s been found the easiest and most reliable r dry materials, such as pure crystalline mpounds and dried pulverised tissues. owever, the method can also be used for uids. For the fusion, an 8 ml semimicro mb[1] has been found suitable. When a great mber of oxidations are to be performed, recommend the use of more than one sion cup with each bomb. The directions r use of the bomb are given in detail in the Parr Manual (1950). However, the final bromine oxidation mentioned there can often be omitted.

The contamination risk can be minimised by careful polishing of the bomb with steelwool, soap and water. Furthermore, every tenth oxidation should be performed on some inactive sulphur-containing material and the barium sulphate from this oxidation assayed for the presence of radioactivity. This will provide a check on the possible contamination of the bomb.

Samples containing high radioactivity should be oxidised in a separate fusion cup.

2) Oxidation with copper nitrate-sodium chlorate, according to Benedict (Hawk, Oser & Summerson 1947). This method has been found convenient for the determination of total sulphur radioactivity in urine specimens. Furthermore, a number of pure compounds can be oxidised in this way with a theoretical yield of barium sulphate. To avoid loss of material due to spurting, we recommend inverting a smaller porcelain dish (10 cm) on top of the oxidation dish (13 cm).

3) Nitric acid-perchloric acid digestion. This is a modification proposed by Rimington (1952) of the method originally described by Boursnell, Francis & Wormall (1946). To a sample in a 50 ml Kjeldahl flask is added 15 ml of a mixture consisting of 3 volumes of concentrated nitric acid and 1 volume of 60 per cent perchloric acid. To this is further added 0.1 g of copper wire. The mixture is heated on an air-bath overnight and then distilled to dryness by increasing the temperature. The recsidue is dissolved in 4 ml of 15 per cent hydrochloric acid and again evaporated to dryness. The residue is then dissolved in water; hydrochloric acid is added and the sulphate precipitated in the ordinary way. For the reagent volumes given above, the amount of protein in the samples should not exceed 0.25 g, or roughly 1 g of soft tissue.

This method is too tedious to be recommended as a routine procedure. However,

[1] Series 2320, 8 ml Semi-Micro Bomb obtained m Parr Instrument Company, Illinois, United ates of America, was found well suited.

it is well suited for samples difficult to oxidise, such as wet tissues, whole blood, serum and other protein-containing solutions.

Precipitation and Ignition. The sulphate from the oxidation mixture is converted to barium sulphate and determined gravimetrically as described by Kolthoff & Sandell (1946).

For the determination of free and total sulphate in urine (the latter after acid hydrolysis of the urine (Hawk *et al.* 1947)), it is recommended that carriers be added for the other radioactive compounds present whenever this is possible. This will decrease the adsorption of non-sulphate radioactivity to the precipitate. To further minimise contamination the sulphate should first be isolated and purified as benzidine sulphate (Hawk *et al.* 1947) before the final barium sulphate precipitation.

The precipitation of barium sulphate is performed at room temperature and left overnight as the microcrystalline precipitate formed under these conditions has been found better suited for planting.

Plating. We have tried several of the methods described for the plating of radioactive samples (Calvin, Heidelberger, Reid, Tolbert & Yankwich 1949). The most reliable procedure was found to be mounting by sedimentation on a cupped planchet. The greater part of the ignited and weighed barium sulphate is transferred to an agate mortar and finely ground with approximately one ml of ethyl alcohol. The suspension is transferred to a cupped nickel-plated planchet by means of a Pasteur pipette. An even distribution is obtained by resuspending the precipitate by means of the pipette and gentle rotation of the planchet. The plate is then left overnight on a horizontal support in a drying oven at 70° C. The amount of barium sulphate plated is determined by weighing the cup before and after the mounting.

Counting. With solid samples of low energy β-emitters, the highest counting efficiency will be obtained with a windowless flow-counter. For routine work we have found it more practical to use a thin window Geiger-Müller counter, since this can be used in connection with an automatic sample changer and recorder. The lower counting efficiency obtained with this type of counter is compensated for by counting the samples over a longer period of time. In our work we have employed a Tracerlab Autoscaler which operates on a pre-set number of counts ("count controlled operation). As the standard setting for the scaler, we have chosen 1024 counts, since this will give a reasonably low standard deviation for a single determination, without resulting i an unduly long counting time for the background. The number of single rounds recorded for each sample will depend on the degree of precision one wishes to obtain in a particular experiment.

Rejection of suspect values. When the recorded values, *i. e.*, times required to reach the pre-set number of counts, are tabulated one may find that for a particular plate some of the values differ greatly from the mean value. Values which are obviously erroneous can be excluded immediately. To determine whether a value within a series should be included in the mean, the use of the Chauvenet criterion may prove helpful. Tables for permissible deviation based on this criterion can be found in the literature (Calvin *et al.* 1949). For "count controlled" operation a graph relating permissible deviation to number of rounds counted is most convenient (Fig. 1). In cases where the counting has proceeded over a long period of time, the limits for the permissible deviation have to be extended to make allowance for the decay of S^{35}.

Correction of counting data. After the mean value for the counting rate of a sample has been calculated, several corrections have to be applied:

1) Coincidence correction. This corrects for errors resulting from the resolving time

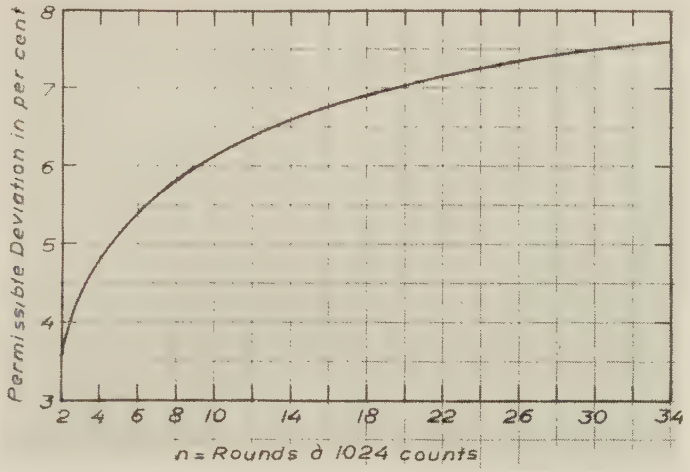

Fig. 1. Permissible deviation according to the "Chauvenet Criterion" as a function of the number of rounds à 1024 impulses registered.

f the counter. As a first approximation, ıe following formula can be used:

$$\frac{N}{R} = \frac{1}{1-T.R.} \quad \text{(Taylor 1951)}$$

here R is recorded counting rate, N is true ɔunting rate and T is resolving time of the ɔunter in units corresponding to R and N. graph relating correction factor (N/R) counts per minute (c. p. m.) is recom-ended.

2) Background correction. The value ob-ined for the counting rate of an empty cup subtracted from the value obtained for the mple after correction for coincidence. This ves the "net counting rate".

3) Correction for decay. Since it is often ɔt possible to count all the samples from ı experiment on the same day, the decay the radioactive sulphur has to be taken to account. Most commonly the start of ıe experiment is taken as the "zero date" ıd all counting data are corrected back to is date. A graph for decay can easily be ɔnstructed from the known value for the lf life.

Decay correction can also be made by counting a standard sample of radioactive barium sulphate along with each set of cups, and then correcting all counting rates against this standard. In this way one also gets a correction for possible changes in the counter efficiency. A combination of these two methods for decay correction will give the best check on the performance of the counter.

4) Correction for self-absorption. For low energy β-emitters, such as C^{14} and S^{35}, the self-absorption in the sample becomes significant even for very thin layers of material. In theory the correction curve for self-absorption plotted against layer thickness should be logarithmic. Therefore, it should be possible to construct the correction curve from a few measurements of samples with the same specific activity but with various layer thickness (Schweitzer & Stein 1950). In practice, other effects, such as "back-scattering", interfere and it seems that the only practical solution is to determine the self-absorption curve from a rather large series of plates made up from the same batch of radioactive material and covering

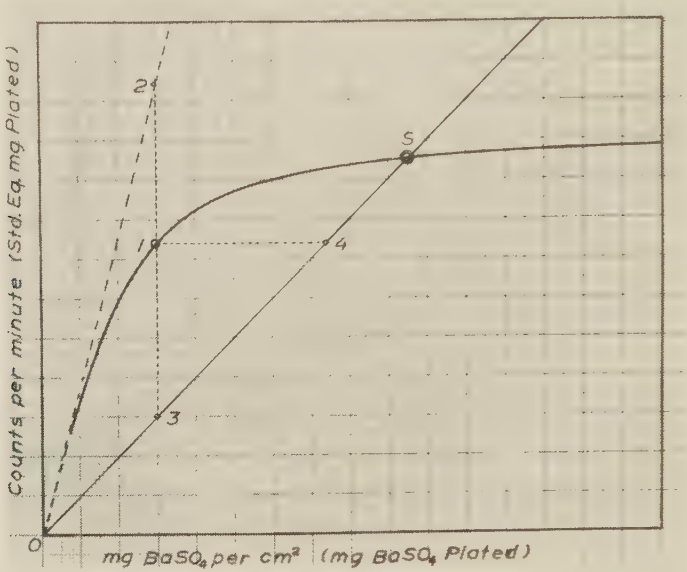

Fig. 2. Self absorption curve for $BaS^{35}O_4$, determined from a series of plates made up from the same batch of radioactive material. The three principal ways of correcting for self absorption are indicated.

the expected working range of plate thicknesses.

Many workers have tried to avoid the correction for self-absorption by plating samples of either "infinite thickness" or "infinitesimal thickness". We have based our technique on plate thicknesses in the range of 5 to 30 mg/cm². Preferably cups of 15 to 25 mg/cm² are prepared, but our experience is that cups of lower layer thicknesses will often occur, for instance, when a sample contains less sulphur than originally anticipated.

A typical curve for counting rate plotted against plate thickness is given in Fig. 2. In correcting for self-absorption there are several possibilities. One is to correct the measured values to the values one would get if no self-absorption occurred, *i. e.*, from point 1 to point 2. Another possibility is to define a plate of specified thickness as a "standard plate" and correct all measurements against this standard, *i. e.*, to make the observed values conform to the straight line O—S. This can be done either by correcting the counting rate to a "standard equivalent counting rate", *i. e.*, from point 1 to point 3, or by correcting the weight of the material on the plate to a "standard equivalent weight", *i. e.*, from point 1 to point 4. In the latter method, which is the one we have chosen, the ordinate on Fig. 2 can be renamed "standard equivalent mg plated" and the units chosen so that point S reads the same on both axes. From the curve we can then, for any amount plated, obtain the corresponding "standard equivalent mg plated", which is the value used for the calculation of the specific activity.

Counting efficiency and absolute counting rate. From a sample of known absolute disintegration rate we have found that when counting under our standard conditions the efficiency is only approximately 0.7 per cent.

If one knows the efficiency of a particular arrangement, it is possible to convert the

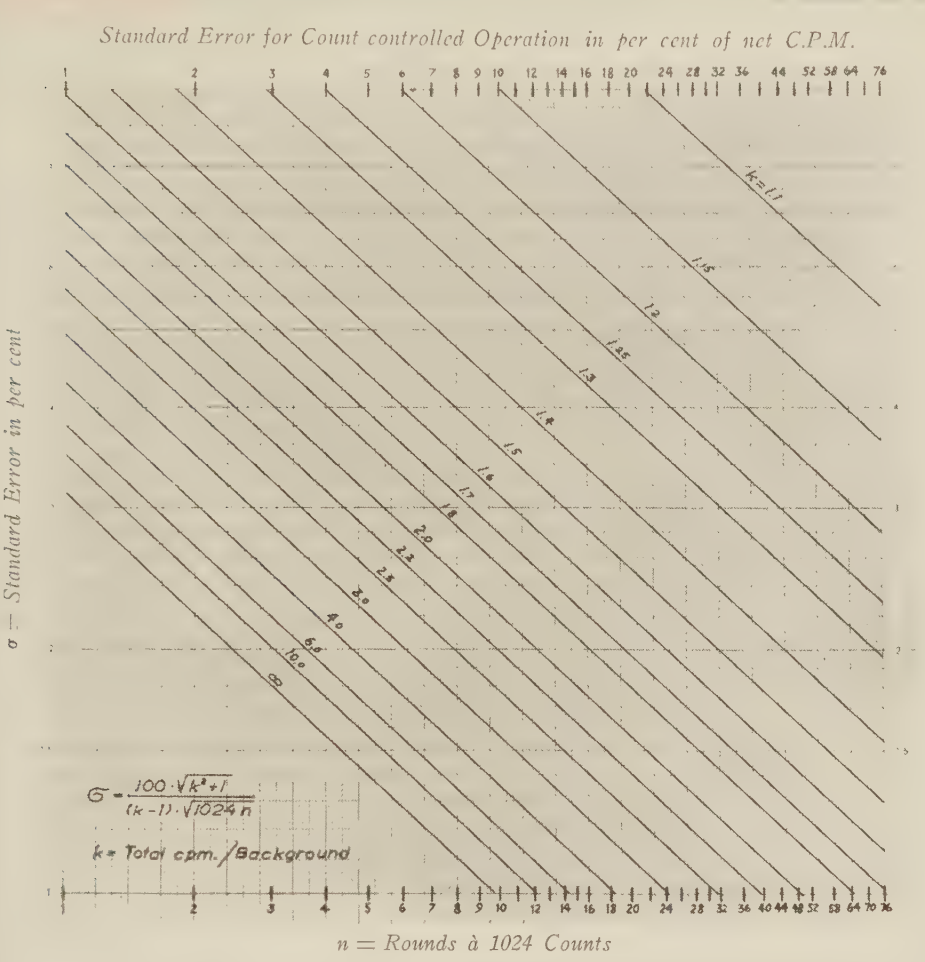
Standard Error for Count controlled Operation in per cent of net C.P.M.
σ = Standard Error in per cent
n = Rounds à 1024 Counts
k = Total cpm./Background
σ = 100 · √(k²+1) / (k-1) · √1024 n

Fig. 3. Standard error plotted as a function of number of rounds à 1024 impulses (abscissa). The k for the parallel lines is the ratio of counting rate for the sample plus background to counting rate of background alone.

ıta to absolute counting rates, and hence to ɔsolute activities in terms of μc. This might e of use when comparing data with those ɔtained in other laboratories or when coun-rs of different efficiencies are being used. he determination of absolute activity is of ajor importance and should always be rried out when deciding the "safe tracer ɔse" in experiments on humans.

Statistical treatment of counting data. Calvin *et al.* 1949, Taylor 1951.) To avoid e tedious calculations involved in deter-mining the standard error of the counting rates, one can make nomograms which are fairly simple to construct if one is using either a "count controlled" or "time controlled" operation. For a "count controlled" operation, such as we are using, the standard error can be expressed, in per cent of *net* counting rate as:

$$\sigma = \frac{100 \times \sqrt{k^2+1}}{\sqrt{N \times (k-1)}} \quad \text{(Taylor 1951)}$$

EXPERIMENT: _____ SAMPLE N° _____ PLATE N°

1. SAMPLING & PLATING DATA:
Total sample...(wt. or vol.)= _____ (A)
Aliquot oxidized(")= _____ (B)
Carrier added: _____
Tot.$BaSO_4$: Gross=
Tare = _____
Net = ... = _____ (C)
$BaSO_4$ plated: Gross=
Tare = _____
Net = _____ (D)
Standard Equivalent mg plated _____ (E)
Condition of plate:

2. COUNTING DATA:
Date counted:
Zero date: _____
Decay, days = _____
Decay factor = _____ (F)
Total cpm..... = _____ (G)
[illegible] = _____ (H)
Corrected tot. cpm.=G x H = _____ (J)
Background cpm..... = _____ (K)
Net. cpm.....=J - K = _____ (L)
Corrected net cpm.= L/F = _____ (M)

3. SAMPLE ACTIVITY DATA:
Std. cpm. pr. mg sample = $\frac{M \times C}{E \times P}$ = _____ (N)
Std. cpm. pr. total sample = N x A = _____

4. STANDARD ERROR CALCULATION:
Ratio tot. cpm/Background = J/K = _____ =k
N° rounds recorded(a 1024 ct) _____ =n
Std. error of net cpm. _____ %

6. ABSOLUTE ACTIVITY OF SAMPLE:
Abs. cpm. pr. mg sample = N/R = _____
μc S^{35} pr. mg sample = N/P = _____

5. COUNTING EFFICIENCY:
Standard cpm. pr. μc S^{35} = _____ (Q)
Absolute cpm. pr. μc = 2,220,000 (P)
Counting efficiency=P/Q = _____ (R)

Fig. 4. The form used in this laboratory for tabulation of the data for a sample through the complete analysis procedure for S^{35}-radioactivity.

where k is the ratio of counting rate for the sample plus background to counting rate of background alone, and N the number of counts made. If we plot log σ against log N, wew ill get a series of parallel straight lines with a slope of $-1/2$ for various chosen values of k (Fig. 3). This graph is also useful in determining the number of counts (or number of rounds) one will have to record to obtain a predetermined statistical error when the value of k is known.

We have here considered only the statistics of the counting operation. For the final calculated values for specific activities other errors will often become of greater significance; for example, there may be weighing errors, losses due to incomplete oxidation or occurring during transfer, uneven plating, *etc.* It is of great interest to have some idea of the reproducibility not only of the counting but also of the other operations involved. For example, for a series of 15 urine samples we have found the following values for the "average spread" between duplicate analyses: free sulphate, 8.6 per cent; total sulphate, 7.9 per cent; total sulphur, 5.5 per cent. The counting rates in this series were determined with less than 1 per cent standard error. For each type of analysis and for each modification of a method previously used the overall error must of course be individually determined.

Tabulation of data. For the final calculation of the radioactivity in a particular

mple, it is most convenient to have all data rtaining to the sample tabulated on a single rm. This form can be designed so that it llows the sample chronologically through e complete analysis procedure. All for-ılas for the required calculations should given to minimise the chance of system-c errors. The form shown in Fig. 4 has en designed for use with samples contain-g radioactive sulphur when counted as rium sulphate as described in the present mmunication.

THE DETERMINATION OF RADIO-ACTIVITY DOSAGE IN HUMANS

The amount of radioactivity which can fely be administered to humans is still an ısettled question. However, it is a generally cepted rule that no organ must receive ore than 0.05 rep of radiation within the rst 24 hours. The dosage to each organ ll depend on the organ distribution and cretion of the material. From studies on perimental animals it is possible to deter-ne whether the administered radioactivity tends to concentrate in a particular tissue. If it does concentrate in certain tissues the dose wil have to be reduced correspondingly. On the other hand, rapid excretion of the radioactivity will permit an increase in the administered dose. The "safe tracer dose" thus calculated represents the upper limit of administered radioactivity. The lower limit must be estimated according to the type of experiment planned and the counting technique to beused. Using the techniques described above, we have been able to carry out experiments on humans with as little as 3 μc of radioactive sulphur.

SUMMARY

We have presented some comments on the techniques we are employing for the assay of S^{35} in biological samples. Some remarks on dosage levels of radioactivity in tracer work on humans have also been included.

ACKNOWLEDGMENT

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PROCEDURE

ursnell, J. C., Francis, G. E. & Wormall, A.: Studies on mustard gas ($\beta\beta^1$-dichlorodiethyl sulphide) and some related compounds. Biochem. J. *40*, 743, 1946.

lvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M. & Yankwich, P. F.: Isotopic Carbon. John Wiley & Sons, Inc., New York, 1949, p. 106, p. 290.

djarn, L.: The conversion of cystinamine to Taurine in rat, rabbit, and man. J. Biol. Chem., *206*, 483, 1954.

awk, P. B., Oser, B. L., & Summerson, W. H.: Practical physiological chemistry. The Blakiston Co., Philadelphia, Pa., U. S. A., 1947, p. 886, p. 884, and p. 887.

lthoff, I. M. & Sandell, E. B.: Textbook of Quantitative inorganic Analysis. The MacMillan Co., New York, N. Y., U. S. A., 1946, p. 329.

Meyer-Schützmeister, L.: Die physikalischen Voraussetzungen für das Arbeiten mit künstlich radioaktiven Substanzen. Naturwiss. *37*, 501, 1950.

Parr Manual Number 121, 1950. Parr Instrument Co., 211 Fiftythird Street, Moline, Ill., U.S.A.

Rimington, C.: Personal communication, 1952.

Schweitzer, G. K. & Stein, B. R.: Measuring solid samples of Low-Energy Beta Emitters. Nucleonics, p. 65, Sepetember 1950.

Taylor, D.: The measurement of radio isotopes. Methuen & Co., Ltd., London, 1951, p. 55, p. 86.

Youden, W. J.: Statistical methods for chemists. John Wiley & Sons, Inc., New York, N. Y., U. S. A., 1951, p. 16.

Young, L. & McCarter, J. A.: The measurement of radioactive sulphur (S^{35}) in Biological material. Biochem. J. *44*, 179, 1948.

Book Review

Methods in Medical Research. Volume 6. Editor-in-Chief: J. M. Steele. Year Book Publishers, Inc., Chicago. Price $ 7.00.

A new volume of this valuable and useful series on Methods in Medical Research has appeared. The following subjects are treated in this volume: Some methods of studying human genetics. Methods in environmental medical research. Design and construction of metabolism cages.

A total of 28 contributors and reviewers have treated a number of different aspects of the above-mentioned fields. The presentation, as in the earlier volumes, is clear and concise and it is illustrated with good figures and diagrams. What A. C. Corcoran wrote of the earlier volumes of the series also holds for this one — "it will find its way into the laboratories rather than the libraries".

S.

Brookhaven Symposia in Biology, No. 5. Major metabolic fuels. Available from the Office of Technical Services, Dept. of Commerce, Washington 25, D. C., price $ 1.35. Also available in book form from commercial publishing houses.

This volume is the record of a symposium held in September 1952 by the Biology Department, Brookhaven National Laboratory. The various lectures were divided into four sections and included: The rate of glucose utilisation; session chairman: de Witt Stetten, Jr. — Hormonal Controls; session chairman: Frank Dickens. — New Metabolic Pathways; session chairman: Bernard L. Horecker. — Fatty acids and ketone bodies; session chairman: Fritz Lipmann.

The introductory address on "The production, disposal and regulation of the blood glucose" was given by S. Soskin. In his detailed and extensive review he stressed the need for caution in th interpretation and application of data from *in vitr* experiments. And he says "at the present time ou judgment as to what the cell does as opposed t what it can do, must be guided by the results of th physiologists and biochemists working with intac living animals, organs or tissues".

S.

Sigurd Funder: *Practical Mycology* Manual for Identification of Fung A. W. Brøgger's Boktrykkeri, Oslo Price: N. Kr. 38.50.

A beautiful little book with excellent illustration designed to serve as an introduction to mycolog for beginners and as an aid for those who, withou any special botanical knowledge, are interested i identifying the most common fungi they encounte in the course of their work, *e. g.* physicians, bio chemists, pharmacists, etc.

S.

F. Wuhrmann & Ch. Wunderly: *Di Bluteiweisskörper des Menschen.* II Edition, 1952. Benno Schwabe Basel. Price: Fr. 42.—.

The second edition of this book, which firs appeared in 1947, represents an extensive revision This excellent study of blood proteins covers thei chemistry, different methods of study includin electrophoresis, paper chromatography, ultracentri fugation, spectrophotometry, etc. and their clinica significance with special reference to diseases wit quantitative and qualitative abnormalities.

The book is very well planned and has numerou good illustrations, figures and diagrams. It wil undoubtedly be one of the most important referenc works on the subject.

This second edition is also translated into Eng lish, Spanish and Italian.

S.

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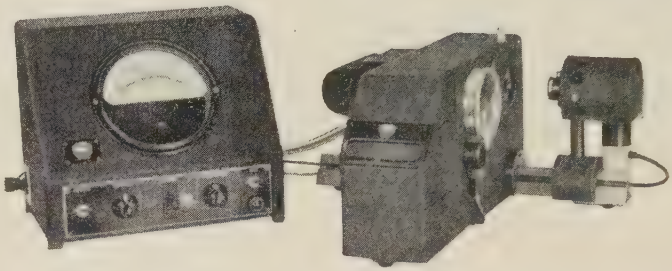

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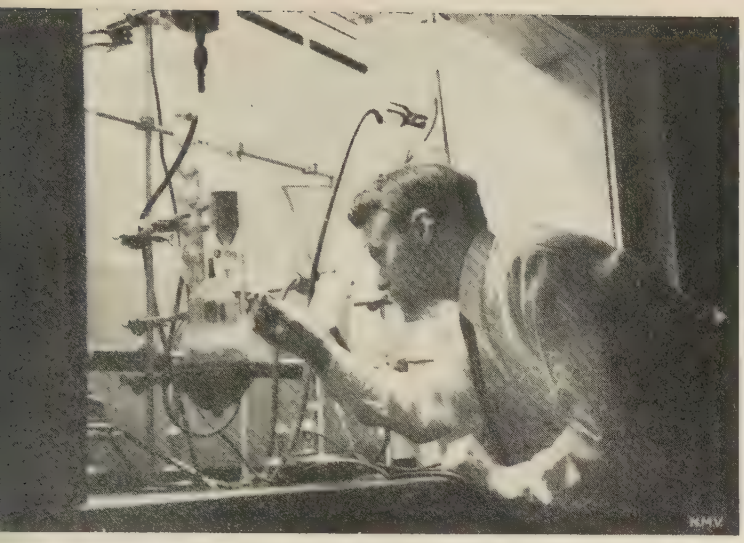

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